ROBISON, CHARLES ELLIOT, Ph.D. The Relationship between Oxidized Low-Density Lipoprotein and Paraoxonase 1 Following Acute Exercise. (2011)
Directed by Dr. Paul Davis.149 pp.

Heart disease and stroke cause significant death and disability in industrialized nations. Oxidized low-density lipoprotein (ox-LDL) is an essential component of the plaque formation within arteries that leads to these conditions. Paraoxonase 1 (PON1) is an antioxidant enzyme capable of preventing the oxidation of LDL. The purpose of this study was to measure the relationship between ox-LDL concentration and PON1 activity in response to acute exercise. Fifteen aerobically trained individuals (M=12, F=3, $VO_{2\ max}$= 54.8 ± 1.7ml·kg^{-1}·min^{-1}, BMI = 22.9± 0.5kg·m^{-2}) completed separate exercise treatment sessions at 60% and 80% $VO_{2\ max}$ for 30 minutes. Ox-LDL concentration and PON1 activity were measured immediately before (PRE), immediately post (0 POST), and 15 minutes post (15 POST) exercise. To ascertain the degree of oxidative stress induced by the exercise sessions, protein carbonyl (PC) concentration and total antioxidant capacity (TAC) were measured at PRE and 0 POST. Repeated measures analyses of variance revealed that ox-LDL concentration and PON1 activity failed to change significantly in response to both the 60% and 80% treatment sessions. In addition, Pearson product moment correlations found no significant relationships between the changes in ox-LDL and PON1 at any time point ($p>0.05$). There were no significant changes in PC or TAC from PRE to 0 POST in either treatment. The results from this study indicate that thirty minutes of running at 80% $VO2_{max}$ was not sufficient to induce oxidative stress and elevate ox-LDL concentrations and PON1 activity in these highly trained subjects.

THE RELATIONSHIP BETWEEN OXIDIZED LOW-DENSITY LIPOPROTEIN AND PARAOXONASE 1 FOLLOWING ACUTE EXERCISE

By

Charles Elliot Robison

A Dissertation Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
2011

Approved by

_____
Committee Chair

To my grandmother, Velma Robison, whose dedication

to a physically active lifestyle has been an inspiration.

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School of The University of North Carolina at Greensboro.

Committee Chair_____

Committee Members_____

_____

_____

_____
Date of Acceptance by Committee

_____
Date of Final Oral Examination

ACKNOWLEDGMENTS

The completion of this dissertation would not have been possible without the generous support of Paul Davis and the members of the committee. I must also thank my loving wife, Kayla, for her patience and encouragement throughout this process.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in the industrialized world. Heart disease and stroke are major forms of CVD and are the first and third causes of death in the United States, respectively (Xu, Kochanek, Murphy, & Tejada-Vera, 2010). Atherosclerosis, the underlying cause of most heart attacks and strokes, is characterized by the development of arterial plaque. The development of atherosclerosis involves several complex processes, including the infiltration and oxidation of low-density lipoprotein (LDL) within the arterial wall. Oxidized LDL (ox-LDL) has been shown to be a major contributor to the dangerous accumulation of lipids beneath the vascular endothelium. Within the intima the ox-LDL particles can be engulfed by macrophages, which in turn comprise the core of arterial plaque.

In contrast, the human body has the capacity to protect itself from the harmful effects of ox-LDL through a variety of mechanisms. In particular, antioxidants in the form of enzymes, vitamins, and other reducing agents have the capacity to disrupt oxidation processes, and thus prevent LDL oxidation. The balance between pro-oxidants and antioxidants plays a key role in the oxidative modification of LDL. Paraoxonase 1 (PON1) is a circulating antioxidant enzyme that is bound to the surface of high-density lipoprotein (HDL). PON1 activity has been shown to have a significant inverse

relationship with several oxidative stress-related diseases such as type II diabetes (B. Mackness, et al., 1998), metabolic syndrome (Senti, et al., 2003), and coronary artery disease (B. Mackness, et al., 2003). In addition, PON1 has been reported to prevent the oxidation of LDL *in vitro* (Camps, Marsillach, & Joven, 2009).Therefore, the action of this enzyme has significant implications in the attenuation of oxidative stress and the prevention of CVD. It is important to understand how the antioxidant system functions to attenuate the oxidation of LDL. However, the *in vivo* dynamic between ox-LDL and PON1 is poorly understood.

Increased oxygen consumption during physical exercise contributes to elevated reactive oxygen species (ROS) generation. The increased ROS concentration can then lead to elevated ox-LDL concentration within the circulation. Although exercise training may reduce circulating ox-LDL, an *isolated* bout of exercise can transiently increase ox-LDL concentration, with values returning to baseline within hours (Tomas, et al., 2002). This feature of exercise provides an opportunity to examine the paired response of ox-LDL concentration and PON1 activity during this time of disrupted ox-LDL homeostasis. To date, the explicit measurement of the relationship between ox-LDL and PON1 following physical activity has not been studied. An examination of this kind should shed new light on the *in vivo* link between these two substances. In addition, it has been observed that the intensity of exercise can affect oxidative stress, but this phenomenon has not been studied with ox-LDL or PON1. More information about the relationship between ox-LDL and PON1 can be gathered by examining these substances after exercise bouts of different intensities.

Purpose of the Study

The purpose of this investigation was to examine the association between ox-LDL concentration and PON1 activity in response to acute exercise. Additionally, this study was designed to measure the influence of oxidative stress, as induced through differing exercise intensities, on ox-LDL concentration and PON1 activity.

Specific Aims

Specific aim #1: Investigate the effect of acute exercise on ox-LDL concentration in response to two acute exercise treatments of different intensities.

Hypothesis 1: Ox-LDL concentration will increase in response to acute exercise to a greater extent with the higher exercise intensity.

Specific aim #2: Investigate the effect of acute exercise on PON1 activity in response to two acute exercise treatments of different intensities.

Hypothesis 2: PON1 activity will increase in response to acute exercise to a greater extent with the higher exercise intensity.

Specific aim #3: Examine the relationship between changes in ox-LDL concentration and PON1 activity in response to two acute exercise treatments of different intensities.

Hypothesis 3a: A significant correlation will exist between exercise-induced changes in ox-LDL concentration and PON1 activity in response to the relatively low exercise treatment.

Hypothesis 3b: A significant correlation will exist between exercise-induced changes in ox-LDL concentration and PON1 activity in response to the relatively high exercise treatment.

Significance of the Study

Ox-LDL and PON1 represent two opposing substances in the progression of atherosclerosis. Although research related to PON1has increased over the past two decades, the role of PON1 in the human body has not been fully described. By measuring the changes of PON1 activity in response to exercise, an oxidative stress stimulant, new information concerning the utility of PON1 can be obtained. One of the possible functions of PON1 is to act as a primary antioxidant upon the rapid increase of ox-LDL. It is plausible that the activity of PON1 is up-regulated in conjunction with a rise in circulating ox-LDL; however this relationship has not been investigated. This study was *significant* because it was the first attempt to quantify the activity of PON1 in relation to the amount of ox-LDL following physical activity, shedding new light onto the possible role of PON1 *in vivo*. The relationship between PON1 and ox-LDL was further defined through the use of differing exercise intensity treatments, with each treatment hypothesized to produce different levels of oxidative stress, ox-LDL, and PON1.

Limitations

1. Participants volunteered for this investigation, thus, a random sample was not taken.
2. Dietary intake was not directly controlled.

3. The measurement of ox-LDL and PON1 was taken from the blood; however the relationship between these two substances may not be the same in the circulation as it is in the arterial intima.

Delimitations

1. Participants completed three day dietary intake logs prior to each experimental treatment. Participants fasted for 12 hours before each exercise treatment to control for dietary influences on blood lipid concentrations.
2. Participants completed one-week physical activity recall questionnaires prior to each experimental treatment.
3. To prevent possible carry-over effects, participants were instructed not to exercise for 48 hours prior to each exercise treatment.
4. Participants were asked otherwise to exercise normally between exercise treatments to prevent a detraining effect.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Lipoproteins are the primary carriers of lipids in the blood. The shape and solubility of lipoproteins confer the properties that allow them to transport insoluble lipids within the water-based blood pool. These spherical, insoluble molecules are surrounded by a coating of proteins and phospholipids that are amphipathic; that is, they have both hydrophobic and hydrophilic regions. The hydrophilic region faces outward while the hydrophobic region faces inward. The core of these particles contains primarily cholesterol ester and triglyceride. The outer phospholipid-monolayer is diffusible in the blood and allows the delivery of cholesterol and triglycerides to the various regions of the body. The proteins embedded within the phospholipid monolayer (apolipoproteins) confer specificity to the lipoprotein complex, allowing them to be recognized by specific receptors on cell surfaces. Apolipoproteins also stimulate certain enzymatic reactions, which in turn regulates the lipoproteins' metabolic function. The cholesterol, triglyceride, free fatty acid, protein, and phospholipid composition of lipoproteins varies widely, but the general structure is consistent. There are five types of lipoproteins which are typically classified by their density. In order of increasing density, the five types are chylomicrons,

very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL).

Cholesterol Transport

After ingestion of food, triglycerides (TG), cholesterol, and phospholipids undergo digestion primarily in the small intestine through the action of bile salts and pancreatic lipase. Digestion hydrolyzes TG into free fatty acids, monoglycerides, and glycerol. The lipids are aggregated into particles called micelles. Micelles' very small diameter allows them access into the intestinal wall and into epithelial cells. In the endoplasmic reticulum of the epithelial cell, TG are reconstituted and, along with absorbed cholesterol and phospholipid, encased into globules with an apolipoprotein B-48 and phospholipid coating. These particles, called chylomicrons, are released from the epithelial cells into the lymphatic circulation (Hussain, Fatma, Pan, & Iqbal, 2005). Chylomicrons eventually enter the great veins in the neck through the thoracic duct. Once in the circulation, HDL transfers apo E to the surface of chylomicrons.

Chylomicrons are the primary carriers of exogenous lipids, with their lipid composition being mostly TG. The fate of these lipoproteins can follow one of two pathways. One mechanism for chylomicron removal is via lipoprotein lipase (LPL) activity. LPL is an enzyme found in most tissues in the body, but primarily in the capillary endothelium of adipose, cardiac, and skeletal muscle tissue (Zechner, 1997). As chylomicrons pass through the capillaries of these tissues, LPL catalyzes the hydrolysis and removal of TG from chylomicrons. The free fatty acids and diacylglycerols are

quickly taken up by the tissue cells. The loss of TG from chylomicrons increases its density and results in the formation of chylomicron remnants (Hultin & Olivecrona, 1998). The liver cells express receptors for apoE, enabling them to recognize and metabolize chylomicron remnants as they pass through the hepatic circulation. Another mechanism for chylomicron removal is directly through the liver (Redgrave, 2004). Some chylomicron particles travel through the circulation unaffected by LPL. In this case, they are metabolized directly by hepatic cells. The liver is highly capable of using lipid from chylomicrons and chylomicron remnants to produce other lipoproteins, energy, or bile salts. The lipid portion is hydrolyzed in the hepatocyte to free fatty acids, glycerol, monoacylglycerols, diacylglycerols, and cholesterol, but resynthesis of these compounds occurs in a manner similar to the events in the intestinal epithelial cell. Fatty acids entering the hepatocyte can also be used as a substrate for ATP production. The cholesterol portion of lipid delivered to the liver can either be converted to bile salts and secreted in the bile or incorporated into VLDL or HDL and released into the plasma.

The liver can resynthesize TG and, in combination with phospholipid, cholesterol, and protein, form VLDL and HDL. VLDL's primary lipid constituent is TG (its primary apolipoprotein is apoB-100), therefore its primary role is to deliver endogenous TG to peripheral tissues (Shelness & Sellers, 2001). LPL on the capillary endothelium acts on VLDL just as it does on chylomicrons; TG is hydrolyzed and removed from VLDL and entered into the surrounding cells. The catabolism of VLDL results in the transient formation of IDL. IDL is quickly catabolized via LPL and hepatic lipase (HL) and gives rise to the less-buoyant LDL (Havel, 1984).

LDL's primary component is esterified cholesterol. Therefore, its function is to transport cholesterol to various tissues throughout the body. The systemic tissues utilize cholesterol for membrane formation and sex hormone construction. LDL interacts with LDL receptors on cells via its apoB-100, an event that culminates in the removal of the lipoprotein from the circulation (Schneider, 1991). Upon interaction between apoB-100 and the LDL receptor, the lipoprotein is internalized via endocytosis. The number of receptors synthesized by cells varies according to cholesterol requirements.

Opposing the cholesterol delivery role of LDL is the HDL fraction of serum lipoproteins. HDL is the smallest of the lipoprotein particles. They are the densest because of their high proportion of protein. An important function of HDL is to collect unesterified cholesterol from peripheral cells, including lipid-laden macrophages, and other lipoproteins where it may have accumulated and return the lipid to the liver for excretion in the bile. This phenomenon is called reverse cholesterol transport and is traditionally believed to be the main explanation as to why HDL is cardioprotective.

The formation of HDL is initiated by the secretion of its main apolipoprotein, apoA-1. Approximately 2/3 of the apoA-1 is synthesized and released from the liver while the intestine is responsible for about 1/3 (Rye & Barter, 2004). Adenosine triphosphate binding cassette transporter A-1 (ABCA-1) is a ubiquitously expressed cellular lipid transport protein that promotes efflux of phospholipid and free cholesterol from cells to lipid-poor apoA-1. ABCA-1 can be found on the surface of the liver, intestines, macrophages, and a variety of peripheral tissues (N. Wang & Tall, 2003). The lipidation of lipid-poor apoA-1 via ABCA-1 results in the formation of nascent, or pre β,

HDL, however this HDL is discoidal and the lipid accounts for only a small percentage of its mass. Additional cholesterol is sequestered through the activity of the enzyme lecithin: cholesterol acyltransferase (LCAT). LCAT is a plasma enzyme that associates with HDL and, through the catalytic activity of apoA-1, esterifies free cholesterol from the plasma membrane of other cells and the surface of other lipoproteins (Sviridov, Hoang, Sawyer, & Fidge, 2000). The esterification of free cholesterol makes it more hydrophobic and draws it to the core of the nascent HDL making it a more spherical, mature HDL particle.

HDL delivers its cholesterol mostly to the liver by direct and indirect pathways (Groen, Oude Elferink, Verkade, & Kuipers, 2004). The direct HDL removal pathways involve HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. The indirect pathway is mediated by cholesteryl ester transfer protein (CETP). This protein exchanges TG from apoB containing particles (VLDL, chylomicrons, LDL) for the cholesteryl esters of HDL. Chylomicrons deliver cholesterol back to the liver and VLDL is then processed to LDL which can be removed from the circulation by the LDL receptors in the liver.TG is not stable in HDL, but is degraded by HL so that finally small HDL particles are left which restart the uptake of cholesterol from cells.

The net effect of these properties of HDL is the retrieval of cholesterol from peripheral cells and other lipoproteins and its return, as cholesteryl ester, to the liver where it can be catabolized and excreted in bile. Reverse cholesterol transport benefits the cardiovascular system by reducing the amount of deposited cholesterol in the vascular endothelium, thus reducing the risk of fatty plaque formation and atherosclerosis. This

delivery of cholesteryl ester to the liver presumably explains the correlation of high HDL levels with reduced risk of CVD (M. Wang & Briggs, 2004).

Oxidized Low Density Lipoprotein

Ox-LDL and atherosclerosis.

Atherosclerosis is a form of arteriosclerosis in which the thickening and hardening of the blood vessel is caused by the accumulation of lipid-laden macrophages within the arterial wall. Through this process artery walls become narrow and stiff, resulting in reduced blood perfusion. Ischemia caused by atherosclerosis is the principal cause of angina pectoris, myocardial infarction and ischemic stroke. Given that myocardial infarction and ischemic stroke are major forms of heart disease and stroke, atherosclerosis clearly presents a major health problem not only in the United States, but throughout the world (World Health Organization, 2008).

The initiation and progression of atherosclerosis consist of very complex processes that involve several biological phenomena, such as inflammation, oxidative stress, and metabolic disorders. At the center of this pathological development is ox-LDL (Stocker & Keaney, 2004). The oxidative modification of LDL has been implicated in a number of atherogenic steps in the arterial wall including endothelial dysfunction, macrophage migration, smooth muscle cell proliferation, and the release of inflammatory cytokines.

The vascular endothelium, a single layer of cells which lines the inner surface of blood vessels, is vital to the health and function of the circulatory system. The genesis of

atherosclerosis is initiated by an injury to the endothelium of the artery wall. Once endothelial cells become damaged, they can be rendered dysfunctional and unable to release normal amounts of vasodilating and antithrombotic cytokines (Davignon & Ganz, 2004). Potential sources of endothelial insult include hypertension, shear stress, dyslipidemia, smoking, diabetes, and ox-LDL (Pennathur & Heinecke, 2007; Ross, 1993). The altered homeostasis of the endothelium initiates the release of inflammatory cytokines, reactive oxygen species (ROS), and prothrombolitic agents (Libby, 2002; Ross, 1999). The injury also increases endothelial permeability and adhesiveness to monocytes. LDL particles, particularly small, dense LDL, can penetrate into the intima of the artery wall through the site of endothelial injury. If the LDL is not already oxidized in the circulation, it can become oxidized within the intima due to the ROS released from the injured cells and monocyte-derived macrophages (Cathcart, 2004; Harrison, Griendling, Landmesser, Hornig, & Drexler, 2003). The oxidation of LDL is an important step in atherogenesis. The modified LDL particle contains several bioactive lipids capable of exacerbating inflammation and smooth muscle cell migration(Navab, Hama, Anantharamaiah, et al., 2000). In particular, blood macrophage recruitment and infiltration is enhanced (Kita, et al., 1999) where the permeated macrophages engulf injured cells, including ox-LDL. Macrophages have the ability to uptake ox-LDL in an unregulated manner which can lead to the development of lipid-laden macrophages, termed foam cells (Dhaliwal & Steinbrecher, 1999). Foam cells also have the potential to release inflammatory cytokines and ROS, further damaging surrounding endothelial and smooth muscle cells. This vicious inflammatory cycle can continue with more LDL

penetration beneath the endothelium, its subsequent oxidation, and further foam cell activation. The accumulation of lipid-laden foam cells creates fatty streaks below the endothelium. Fatty streaks can enlarge and progress into fibrous plaque that protrudes into the arterial lumen, compromising the delivery of blood to distal tissues. Fibrous plaque can become unstable, rupturing and exposing the underlying tissue to the bloodstream. The ruptured and exposed plaque can then initiate platelet adhesion and the clotting cascade, rapidly forming a thrombus or embolus. The sudden occlusion of the distressed vessel can result in potentially lethal ischemia and infarction. Undoubtedly, the development of this vascular pathology would not take place without ox-LDL, which has been implicated in the initiation and exacerbation of lesion formation. As such, the core of atherosclerotic plaque is primarily comprised of ox-LDL derived lipid (Stary, et al., 1995; Yla-Herttuala, et al., 1989).

The oxidative modification of LDL.

Although not yet proven, most researchers believe that LDL becomes oxidized within the arterial wall (Matsuura, Hughes, & Khamashta, 2008). It is within the subendothelial space that LDL may be exposed to a higher degree of oxidative stress as compared to the circulation. The bidirectional passage of LDL across the endothelium most likely accounts for presence of ox-LDL within the circulation. Under conditions of oxidative stress, several components of the LDL complex can be augmented by ROS. The polyunsaturated fatty acid portion of esterified cholesterol, phospholipids, and triglycerides are the most susceptible to oxidation (Itabe, 2009). The products of ROS induced lipid oxidation can participate in a chain reaction that propagates further lipid

modification. A variety of smaller fragments can be produced, including aldehydes. These aldehyde compounds have the capacity to modify the lysine, cysteine, histidine, and tryptophan amino acids of apoB-100 (Itabe, 2009). The accumulation of oxidized cholesterol, phospholipids, and triglycerides and their subsequent propagation into aldehydes is believed to be the primary pathway by which apoB-100 becomes modified. Another pathway has also been shown to modify apoB-100 in a more direct manner. Myeloperoxidase (MPO), an enzyme released from activated phagocytes, is capable of directly modifying apoB-100-assocated tyrosine (Podrez, Abu-Soud, & Hazen, 2000). The augmentation of the apoB-100 leaves LDL unrecognizable by LDL receptors. However antibodies and phagocytic cells, which are capable of recognizing the modified lipids and proteins of ox-LDL, act to remove the altered particle (Lopes-Virella & Virella, 2010; Shashkin, Dragulev, & Ley, 2005). In addition, ox-LDL has been reported to be cleared from circulation by hepatic Kuppfer cells (Ling, et al., 1997).

Measurement of lipid and protein oxidation and antioxidant capacity.

Exercise and health related studies have employed several types of measurements to assess protein and lipid oxidation and antioxidant capacity of the blood. A brief description of these types of measures is presented below.

Ox-LDL concentration - This is the most direct measurement of circulating ox-LDL. This enzyme linked immunosorbent assay (ELISA) involves the spectrophotometric detection of ox-LDL after it has bound to a microplate labeled for oxidized apolipoprotein B, the dysfunctional form of the principle ligand for LDL receptors.

Ox-LDL lag time - This technique assesses the susceptibility of LDL to oxidation. Isolated LDL particles are subjected to *in vitro* oxidation, usually through a copper ion system. The time to oxidation, usually measured by the development of oxidized lipids, is determined. In this technique, a decrease in lag time (time to oxidation) is an indicator that the LDL particle is more susceptible to oxidation. Conversely, an increase in lag time is an indicator that the LDL particle is less susceptible to oxidation.

Conjugated dienes - When a polyunsaturated fatty acid comes under attack by an ROS a hydrogen atom is removed, resulting is a molecular rearrangement of the fatty acid structure. The result is the formation of a conjugated diene and is considered the initial step in lipid peroxidation. Serum and/or plasma conjugated dienes can be assessed as well as LDL associated conjugated dienes.

Lipid hydroperoxides - A conjugated diene can readily react with an oxygen molecule forming a peroxyl- fatty acid radical. A peroxyl-fatty acid radical is an unstable species and can abstract a hydrogen atom from a polyunsaturated fatty acid forming a conjugated diene and a lipid hydroperoxide. Ultimately, lipid hydroperoxides represent further propagation of the lipid peroxidation process.

Malonaldehyde, ethane and pentane - Lipid hydroperoxides can decompose through a complex process resulting in the formation of fatty acid fragmentation products. These products include aldehydes such as malonaldehyde (MDA) and short chain alkanes such as ethane and pentane. Consequently MDA, ethane and pentane have been assessed as biomarkers for latter stage lipid peroxidation.

Antibodies against Ox-LDL- The modified lipids, phospholipids and proteins associated with ox-LDL are highly immunogenic. The titer of antibodies against oxidized LDL can be evaluated as an indirect biomarker for the presence of ox-LDL.

Protein Carbonyl Concentration – The abundant protein structures found in the blood are also susceptible to ROS attack. ROS interaction with an amino acid can transform the side chain amine group into a carbonyl. Measurement of protein carbonyl concentration within the serum or plasma is commonly used as a general oxidative stress marker.

Total Antioxidant Capacity –The sum of the individual antioxidant substances found in the blood represents the overall ability of the antioxidant system. The antioxidant capacity can temporarily decrease in response to acute exercise as the components of the antioxidant system are used to diminish harmful ROS. Therefore, the degradation of the total antioxidant capacity can be used as an indicator of oxidative stress.

Relationship between circulating ox-LDL and CVD.

As mentioned above, the majority of LDL oxidation likely occurs within the arterial wall, as opposed to the antioxidant-rich environment of the blood (Frei, Stocker, & Ames, 1988). In addition, the proportion of ox-LDL to total LDL is very small, ranging from 0.013% in controls to 0.027% in CAD patients in one study (Holvoet, et al., 2001). Nonetheless, a growing body of evidence indicates a pathological role of circulating ox-LDL in the progression of atherosclerosis.

Several studies have found that a high level of plasma ox-LDL is associated with a number of cardiovascular diseases. Coronary artery disease (CAD) patients, particularly those with acute coronary syndrome (Ehara, et al., 2001; Tsimikas, et al., 2003), have

presented with significantly elevated values of ox-LDL (Holvoet, et al., 2001; Tanaga, et al., 2002). Ox-LDL was also shown to be a prognostic marker for future CAD after heart transplantation (Holvoet, Van Cleemput, Collen, & Vanhaecke, 2000). In addition, atherosclerosis of the carotid artery (Nishi, et al., 2002) and ischemic stroke (Uno, Kitazato, Nishi, Itabe, & Nagahiro, 2003) have been reported to be associated with high plasma concentrations of ox-LDL. Taken together, these results point toward the use of ox-LDL as a biomarker for the presence of advanced atherosclerosis.

Circulating ox-LDL has been identified as a predictor of future cardiovascular events in individuals with documented CVD (Shimada, et al., 2004), as well as re-stenosis following coronary stenting (Naruko, et al., 2006). Compared to the traditional lipoprotein profile and other traditional risk factors, ox-LDL was the strongest predictor of a future coronary event in apparently healthy men (Meisinger, Baumert, Khuseyinova, Loewel, & Koenig, 2005). In addition, a 10 year prospective study found that circulating oxidized phospholipids/apoB-100 ratio predicted cardiovascular events independent of traditional risk factors, highly sensitive C-reactive protein, and Framingham Risk Score (Kiechl, et al., 2007).Other evidence indicates that plasma ox-LDL plays a role in endothelial dysfunction (Penny, et al., 2001) and vasomotor function (Tamai, et al., 1997) in individuals with hypercholesterolemia, but without apparent atherosclerosis.

In a case-control study, Cominacini et al. (2005) assessed the association between ox-LDL and inflammation in patients with angina pectoris. They found that circulating ox-LDL and blood mononuclear NF-κB levels were higher in the patients compared to controls. In addition, the *in vitro* incubation of high dose ox-LDL or serum from the

angina patients increased NF- κB in mononuclear cells. The authors concluded that circulating ox-LDL was responsible, at least in part, for the NF- κB activation, and thus, the elevated inflammation and oxidative stress in the angina pectoris patients. A recent study demonstrated the direct effect of ox-LDL removal from circulation on the development and progression of atherosclerosis (Ishigaki, et al., 2008). Lectin-like ox-LDL receptor 1 (LOX-1) is an ox-LDL receptor found on endothelial cells, macrophages, smooth muscle cells and atherosclerotic lesions in humans (Kume, et al., 1998). LOX-1 is a scavenger receptor that selectively binds and removes ox-LDL from the circulation (Sawamura, et al., 1997). Therefore, to remove circulating ox-LDL, LOX-1 was ectopically expressed in the liver of apolipoprotein E (apoE) deficient mice with an adenoviral gene transfer system. Although plasma total cholesterol, triglycerides and LDL cholesterol were unaltered, plasma ox-LDL was significantly reduced in the hepatic LOX-1 mice compared to controls. In addition, monocyte chemotactic protein-1 and lipid peroxide levels were decreased while adiponectin increased, suggesting a reduction of systemic oxidative stress in LOX-1 mice. Most importantly, LOX-1 expression almost completely prevented atherosclerosis progression, while controls displayed significant atherosclerotic lesions. This investigation dramatically demonstrated the affect of circulating ox-LDL on atherosclerosis development, independent of other blood lipid markers.

Although the extent of the contribution of circulating ox-LDL to atherosclerotic advancement is not fully known, many research studies have shed light on its pathological involvement. Ox-LDL removal is now a therapeutic target in the prevention

of cardiovascular diseases. Investigations targeting the degradation or removal of ox-LDL from circulation are receiving considerable attention as a treatment strategy for atherosclerosis (Ishigaki, Oka, & Katagiri, 2009). More information needs to be collected concerning the receptors and antioxidants responsible for the clearance of ox-LDL from the circulation.

Paraoxonase 1

Paraoxonase 1 (PON1) is a calcium-dependent A-esterase enzyme that is synthesized and secreted primarily from the liver. The name paraoxonase is derived from paraoxon, the first organophosphate (OP) substrate revealed to be hydrolyzed by the enzyme (Aldridge, 1953). Originally, PON1 was investigated due to its ability to hydrolyze toxic OP insecticides such as parathion (metabolized to paraoxon) (Aldridge, 1953; Geldmacher-v Mallinckrodt, Hommel, & Dumbach, 1979; Playfer, Eze, Bullen, & Evans, 1976), chlorpyrifos (Furlong, Richter, Seidel, Costa, & Motulsky, 1989; Furlong, Richter, Seidel, & Motulsky, 1988), and diazinon (Davies, et al., 1996). In addition PON1 was found to be capable of degrading the nerve toxins soman and sarin (Smolen, Eckerson, Gan, Hailat, & La Du, 1991). These features made PON1 an obvious topic of research in the areas of agriculture and biological warfare. Indeed, in animal and human studies PON1 activity was shown to correlate well with OP insecticide and nerve gas sensitivity. Rabbits, which have higher PON1activity than rats, are more resistant to insecticide poisoning than rats (Costa, Richter, Murphy, & Omenn, 1987). Furthermore, the intravenous administration of purified PON1 to PON1 null mice improved protection

against insecticide poisoning (Li, et al., 2000; Stevens, et al., 2008). In Gulf War veterans, low PON1 activity has been shown to be associated with greater neurological symptoms from possible toxic nerve gas exposure (Furlong, 2000; Haley, Billecke, & La Du, 1999). As a result of such studies, PON1 is currently a prime target of research for human medical treatment of insecticide and nerve agent exposure (Boado, Zhang, Zhang, Wang, & Pardridge, 2008; Rochu, Chabriere, & Masson, 2007; Yair, et al., 2008).

More recently PON1 has drawn attention as an antioxidant and anti-inflammatory agent. Low PON1 enzymatic activity has been associated with several disease states that share the common theme of oxidative stress. These pathologies include types 1 and 2 diabetes (Abbott, Mackness, Kumar, Boulton, & Durrington, 1995; B. Mackness, Durrington, Boulton, Hine, & Mackness, 2002; B. Mackness, et al., 1998), chronic renal failure (Dantoine, et al., 1998), uremia (Biasioli, et al., 2003), thyroid dysfunction (Azizi, et al., 2003), rheumatoid arthritis (Tanimoto, et al., 2003),multiple sclerosis (Ferretti, et al., 2005), metabolic syndrome (Sentí, et al., 2003), hyperlipidemia (Paragh, et al., 1998), and coronary artery disease (B. Mackness, et al., 2003).

Since the oxidative modification of LDL is the leading theory describing the relationship between elevated LDL and atherosclerosis, PON1 has recently become an enzyme of great interest in the CVD research. Several studies have demonstrated that PON1 can inhibit the oxidation of LDL and hydrolyze LDL-derived lipids once they are formed (see below). These investigations have led several authors to suggest that the natural physiological function of PON1 is to hydrolyze oxidized lipids, thus serving as a cardioprotective antioxidant (Camps, et al., 2009; James, 2006). The antioxidant capacity

of PON1 has also made it an optimistic target for CVD prevention and treatment (Costa, Cole, Jarvik, & Furlong, 2003; Durrington, Mackness, & Mackness, 2002). Claims concerning PON1's ability to hydrolyze these specific oxidized lipids have not been fully substantiated and needs further study. In addition, the relationship between oxidized LDL and PON1 has not been fully described; information concerning this relationship could prove to be very important in the prevention of CVD.

PON1 level is typically measured as either enzymatic activity and/or protein concentration in the serum. PON1 can hydrolyze several substrates including paraoxon, phenylacetate, diazoxon, and several lactones, all of which have been used to describe PON1 activity (Table 1). PON1's versatility is a feature that researchers have labeled as "catalytic promiscuity" due to its broad range of substrate partners (Bornscheuer & Kazlauskas, 2004; Copley, 2003; O'Brien & Herschlag, 1999). Investigations have utilized this variety of substrates to assess PON1 activity and there is no consensus as to which substrate is more appropriate. PON1 enzyme concentration can also indicate the level of protective potential; however most exercise studies have chosen to assess PON1 quality (activity) as opposed to the quantity (concentration).

PON1 association with HDL.

PON1 is an enzyme with a molecular mass of 43-45 kDa (354 amino acids) (Blatter, James, Messmer, Barja, & Pometta, 1993; Gan, Smolen, Eckerson, & La Du, 1991). It is produced and secreted primarily from the liver. The production and secretion from the hepatocytes is the largest determinant of serum PON1 level. There is considerable variability in the serum concentration and activity of PON1 among

Table 1. Summary of Methods for Determination of PON1 Enzymatic Activity

Substrate	Activity
Paraoxon	Paraoxonase(esterase)
Phenylacetate	Arylesterase
Diazoxon	Diazoxonase (esterase)
Soman	Esterase
Sarin	Esterase
Homocysteinethiolactone	Lactonase
5-thiobutyl butyrolactone (TBBL)	Lactonase
7-*O*-diethyl phosphoryl 3-cyano 4-methyl 7-hydroxycoumarinDEPCyMC	Lactonase

Adapted from Camps, et al(2009).

individuals. These variations are a result, to some extent, of several polymorphisms in the coding and promoter regions of the PON1 gene (Adkins, Gan, Mody, & La Du, 1993; Humbert, et al., 1993; Leviev & James, 2000; Suehiro, et al., 2000). PON1's production leaves the peptide with a highly hydrophobic N-terminal region (Sorenson, et al., 1999). Therefore, it requires the proper vector to be transported within the aqueous environment of the blood. Deakin et al.(2002) demonstrated that the most suitable acceptor of PON1 is a lipid complex, with HDL having the highest affinity for PON1. HDL is believed to transiently bind to the hepatocyte, possibly via SR-B1 (Acton, et al., 1996), where PON1 is transferred to the lipoprotein. Apo A1, the major protein component of HDL, is most

likely responsible for the binding process and is very important for the enzymatic stability of PON1 in the circulation (Deakin, et al., 2002; James, et al., 1998; Sorenson, et al., 1999). Although PON1in the circulation is exclusively bound to HDL, evidence suggests that it is only found on approximately 10% of circulating HDL (Blatter, et al., 1993; Kelso, et al., 1994).

Modulation of PON1 activity.

Drugs.

Most studies assessing the effects of pharmaceutical agents and PON1 were done so using lipid-lowering statins and fibrates. These studies have yielded conflicting results. PON1 activity increased in patients taking statins, gemfibrozil and fenofibrate (Balogh, et al., 2001; Deakin, Leviev, Guernier, & James, 2003; Jarvik, et al., 2002; Paragh, et al., 2003). Deakin et al. (2003) found that simvastatin increased PON1 promoter activity by increasing sterol regulatory element binding protein 2, however these results were contradicted in another study which demonstrated diminished promoter activity after treatments of simvastatin, pravastatin, and fluvastatin (Gouedard, Koum-Besson, Barouki, & Morel, 2003). Patients treated with ciprofibrate, bezafibrate, and gemfibrozil have shown no change in PON1 activity (Durrington, et al., 1998; Turay, Grniakova, & Valka, 2000). In addition, CAD patients utilizing aspirin as a therapeutic agent displayed increased PON1 activity and concentration (Blatter-Garin, Kalix, De Pree, & James, 2003). This could be due to the anti-inflammatory effect of aspirin or the modulation of PON1 production. Human hepatic cells displayed a significant induction of PON1 gene

expression after incubation with aspirin *in vitro* (Jaichander, Selvarajan, Garelnabi, & Parthasarathy, 2008).

Smoking.

Several studies have reported an association between cigarette smoking and low PON1 activity (Ferre, et al., 2003; James, Leviev, & Righetti, 2000; Jarvik, et al., 2002; Senti, et al., 2003). This effect is apparently short-lived, as one study reported a return to control PON1 level 3-24 months after smoking cessation (James, et al., 2000). This finding suggests that cigarette smoke directly effects PON1 activity, which is supported by *in vitro* evidence (Nishio & Watanabe, 1997).

Alcohol Consumption.

Studies involving middle-aged men and postmenopausal women found that moderate consumption of beer, wine, or spirits for 3 weeks increased PON1 activity (Sierksma, van der Gaag, van Tol, James, & Hendriks, 2002; van der Gaag, et al., 1999). Heavy drinking has been shown to adversely affect PON1 activity. Rao et al. (2003) found that heavy drinkers had a 45% lower, but moderate drinkers had a 395% higher PON1 activity compared to non-drinkers. Despite evidence that moderate alcohol consumption can increase PON1 activity, several studies have concluded that alcohol has no effect on PON1 (Ferre, et al., 2003; Sarandol, Serdar, Dirican, & Safak, 2003; Vincent-Viry, et al., 2003).

Diet.

Although the evidence is not conclusive, the research suggests that high fat diets and trans fat diets tend to decrease PON1 activity while unsaturated fat intake tends to

increase PON1 activity. Male and female rats on a high fat diet for 14 weeks had decreased PON1 activity compared to controls (Thomas-Moya, Gianotti, Proenza, & Llado, 2007). Another study found that replacement of dietary saturated fat with trans fat resulted in a 6% reduction in PON1 activity in healthy men and women (de Roos, Schouten, Scheek, van Tol, & Katan, 2002). Data from a 72-hour dietary recall questionnaire showed that consumption of oleic acid, a monounsaturated fatty acid, was associated with higher PON1 activity in 654 men, but only those with a QR or RR genotypes of PON1(Tomas, et al., 2001). In addition, administration of omega-3 polyunsaturated fatty acid capsules to 14 patients with combined familial hyperlipidemia resulted in a 10% increase in PON1 concentration after 8 weeks (Calabresi, et al., 2004). It should also be noted that administration of partially hydrogenated soybean, soybean, palm, or canola oil for 5 weeks revealed no differences in PON1 activity in men and women over 50 (Vega-Lopez, Ausman, Jalbert, Erkkila, & Lichtenstein, 2006).

Antioxidant and PON1 investigations have yielded contrasting results. Consumption of pomegranate juice for 2 weeks increased PON1 activity by 18% in 13 healthy men (Aviram, Dornfeld, et al., 2000). Pomegranate juice was also responsible for increased PON1 binding to HDL in individuals with diabetes (B. Fuhrman, Volkova, & Aviram, 2009). Vitamins C and E were assessed via recall questionnaire with 189 men and revealed that intake of these antioxidant vitamins were significant positive predictors of PON1 activity (Jarvik, et al., 2002). On the contrary, two studies have reported negative associations between fruit and/or vegetable intake (high in vitamins C and E) and PON1 activity (Kleemola, et al., 2002; Rantala, et al., 2002). In addition, Ferre et al.

(2003) found no association between vitamins C and E and beta carotene intake and serum PON1 activity in 388 men and women. Obviously, the interaction between dietary antioxidants and serum PON1 activity necessitates further study.

Hepatic Regulation.

PON1 modulation at the cellular level of the hepatic cells has been examined in a limited number of studies. Although the evidence is small, investigations agree that PON1 gene expression is dependent on the aryl hydrocarbon receptor (AhR), a cytosolic transcription factor. The antioxidants quercetin and resveratrol significantly increased PON1 gene expression via an AhR dependent mechanism after *in vitro* incubation with HuH7 cells (Gouedard, Barouki, & Morel, 2004a, 2004b). PON1 gene expression increased significantly in human hepatoma cells (HepG2) following incubation with aspirin *in vitro* (Jaichander, et al., 2008). The up-regulation of the PON1 gene was associated with induction of AhR gene expression, a suspected receptor of aspirin metabolites. In addition, one investigation reported that activation of transcription factor Sp1 and protein kinase C were involved in PON1 promoter activity in HepG2 cells (Osaki, et al., 2004).

Oxidative Stress.

As mentioned previously, several disease states associated with increased oxidative stress are also associated with decreased PON1 activity. Precisely how this relationship occurs is not clear. Oxidation products could either down-regulate the gene expression of PON1 or negatively affect PON1 within the circulation. One *in vitro* study demonstrated that a variety of oxidized lipids, including ox-LDL, inactivated PON1

activity after their co-incubation (Aviram, et al., 1999). This information implies that PON1 loses its functional ability in the bloodstream upon interaction, and subsequent hydrolysis, of its target. The incubation in this study was performed for 18 hours, which additionally suggests that the degradation of PON1 could be a chronic effect of the oxidized lipids within the circulation. This mechanism, although not conclusive, suggests that systemic degradation of PON1 activity explains the connection between chronic oxidative stress and many pathophysiologies.

Similarly, very little is known about how oxidative stress affects hepatic PON1 synthesis, release, and binding to HDL, features that could also influence PON1 activity. Only one investigation has examined how one of PON1's primary targets, oxidized lipids, affects the hepatocyte itself. Navab et al. (1997) found that incubation of ox-LDL with HepG2 cells for 16 hours reduced PON1 mRNA by 3-fold. Based off of limited evidence, it seems that constant exposure of ox-LDL to PON1 and hepatic cells results in the loss of activity and expression of PON1, respectively. However, what remains to be investigated is the acute phase response of the PON1 protein and the hepatocyte to the sudden increase in ox-LDL concentration.

PON1 and CVD.

In Vitro Studies.

There is a well-established negative relationship between HDL concentration and incidence of CVD. Several prominent studies have shown that individuals with low HDL are more prone to CVD events than individuals with high HDL (ATP-III, 2002). While HDL's primary cardio-protective role is widely attributed to reverse cholesterol transport,

HDL also exhibits anti-inflammatory, antithrombotic, and antioxidant properties that provide cardiovascular benefits (Florentin, Liberopoulos, Wierzbicki, & Mikhailidis, 2008). The antioxidant activity of HDL is largely attributed to the activity of PON1, as a large body of evidence has demonstrated that PON1 disrupts many of the pro-oxidant processes involved in atherogenesis.

Given the established negative association between HDL and atherosclerosis, and encouraged by evidence that HDL could inhibit the oxidative modification of LDL (Parthasarathy, Barnett, & Fong, 1990), Mackness et al. (1991) attempted to further define the interaction between HDL and LDL in atherosclerosis. The HDL-associated enzyme PON1 was suspected to play a part in the antioxidant properties displayed by HDL. LDL that had been oxidized through copper exposure was incubated with native HDL and purified PON1. The researchers found that HDL and PON1 prevented lipoperoxide generation to a similar extent, implying that the PON1 enzyme was involved in the antioxidant effects observed by HDL. This was the first study to indicate that PON1 protected LDL from potentially atherogenic oxidation and thus pointing to its involvement with CVD. The discovery of this feature of PON1 shed new light on thecardioprotective role of HDL. For the next two decades the direction of PON1 research focused primarily on its connection with the oxidation and inflammatory processes thought to mediate the arterial plaque development.

In vitro experiments with LDL and PON1 have typically involved the isolation of LDL, followed by oxidation induced via copper ions or ROS-generated processes. These processes were conducted either with or without the addition of purified PON1.These

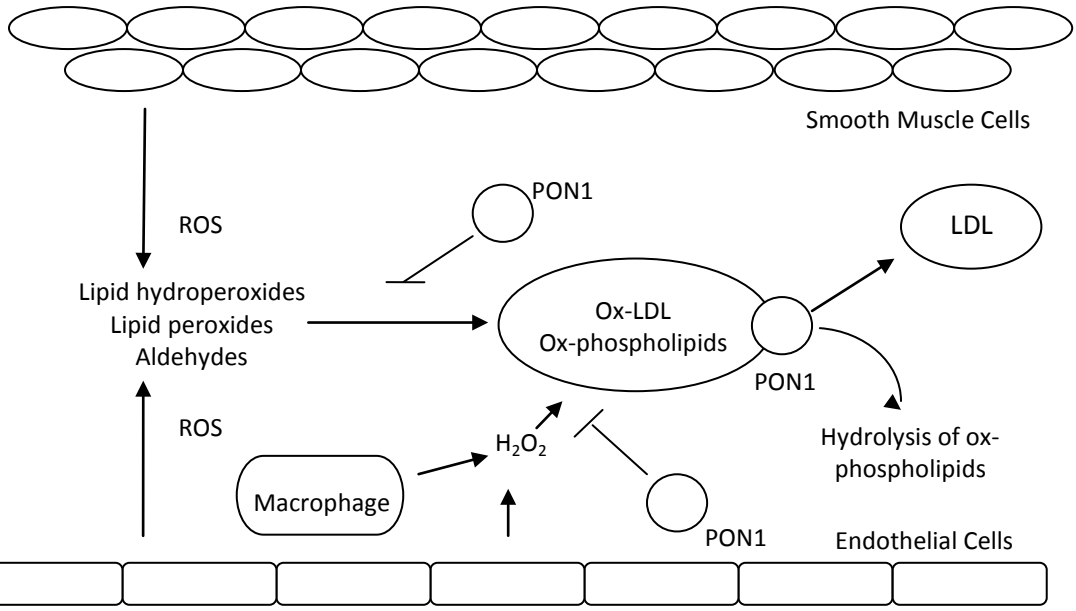
experiments have shown that the addition of PON1 greatly decreased LDL-associated accumulation of oxidized lipids such as lipid peroxides (Aviram, Billecke, Sorensen, et al., 1998; Bianca Fuhrman, Volkova, & Aviram, 2005; B. Mackness, Hine, Liu, Mastorikou, & Mackness, 2004; B. Mackness, Mackness, Arrol, Turkie, & Durrington, 1998; M. Mackness, Arrol, Abbott, & Durrington, 1993), lipid hydroperoxides (Navab, Hama, Cooke, et al., 2000), and aldehydes (Aviram, Billecke, Sorensen, et al., 1998; Bianca Fuhrman, et al., 2005). These oxidized lipids are believed to cause oxidative modification of the phospholipid component of LDL, ultimately modifying the particle'sapoB-100 protein and disrupting LDL function. Specifically, PON1 has been shown to destroy the lipid hydroperoxides hydroperoxyoctadecadienoic acid (HPODE), hydroperoxyeicosatetraenoic acid (HPETE), and cholesteryl hydroperoxide (CE-OOH) (Navab, Hama, Cooke, et al., 2000).In addition, experiments have demonstrated that PON1 can inactivate oxidized phospholipids once they form within the LDL particle, specifically 1-palmitoyl-2-oxovaleryl-*sn*-glycero-3-phosphocholine (POVPC), 1-palmitoyl-2-glutaryl-*sn*-glycero-3-phosphocholine (PGPC), and 1-palmitoyl-2-(5, 6-epoxyisoprostane E2)-*sn*-glycero-3-phosphocholine (PEIPC) (Navab, Hama, Anantharamaiah, et al., 2000; Navab, Hama, Cooke, et al., 2000; A. D. Watson, et al., 1995). Oxidized phospholipids are the primary oxidized lipid found within oxidized LDL and are believed to initiate the inflammatory cell signaling cascade. Essentially, the hydrolyzing action of PON1 toward the oxidized lipids can protect LDL from apoB-100 modification, thus retaining the particle's native, functional state.

Ex Vivo Studies.

In addition, researchers have examined PON1's role in the LDL-endothelial interaction with *ex vivo* cultures of endothelial cells of human carotid, aortic, or coronary arteries. Navab, Hama, Cooke, et al.(2000) found that incubation of LDL particles with PON1 rendered them resistant to oxidation by aortic/smooth muscle cell cultures. Similarly, when aortic cultures where incubated with PON1 prior to LDL introduction, the cultures were incapable of oxidizing LDL (Navab, Hama, Anantharamaiah, et al., 2000). These studies suggest that PON1 reduces ROS generation from the artery wall, which is believed to be the primary avenue by which LDL molecules become oxidatively modified. This antioxidant property was further supported by evidence that PON1 can hydrolyze hydrogen peroxide (Aviram, Rosenblat, et al., 1998), a major ROS produced by endothelial cells and phagocytes under oxidative stress. Evidence has also shown that PON1 can reduce cholesteryl ester hydroperoxides and cholesteryl ester peroxides from carotid and coronary artery atherosclerotic lesions (Aviram, Hardak, et al., 2000). Figure 1 demonstrates the theoretical protective mechanisms by which PON1 preserves LDL from oxidation.

The PON1-induced blockage of LDL oxidation has also been shown to disrupt further steps in foam cell formation. After mildly oxidized LDL or artery wall cultures were incubated with PON1, they were unable to induce monocyte chemotaxis and binding to endothelial cells (Navab, Hama, Anantharamaiah, et al., 2000; Navab, Hama, Cooke, et al., 2000; A. D. Watson, et al., 1995). This inhibition of monocyte cell signaling was apparently caused by the ability of PON1 to inhibit the biological

Figure 1. PON1 Prevention of LDL Oxidation



PON1 Prevention of LDL Oxidation.PON1 can prevent the oxidation of LDL from hydrogen peroxide (H_2O_2), lipid hydroperoxides, lipid peroxides, and aldehydes. PON1 can also hydrolyze oxidized phospholipids within oxidized LDL (ox-LDL). Although depicted within the artery wall, this process is believed to occur in the circulation as well. Adapted from Camps, et al.(2009) and Navab, Hama, Anantharamaiah, et al.(2000).

activity of oxidized phospholipids (Navab, Hama, Anantharamaiah, et al., 2000; A. D. Watson, et al., 1995) which in turn, inhibited NF-κB activation and the production of cytokines (e.g. IL-8, M-CSF), chemokines (e. g. MCP-1), and adhesion molecules (e. g. VCAM-1, ICAM-1) (Aviram & Rosenblat, 2004; B. Mackness, et al., 2004; Navab, et al., 2001; Negre-Salvayre, et al., 2006).

In addition to attenuating foam cell generation through prevention of LDL oxidation, PON1 may also prevent *HDL oxidation* and aid in its reverse cholesterol

transport function. Aviram, Rosenblat, et al. (1998) found that HDL-associated PON1 was very important in the maintenance of the lipoprotein's cholesterol accumulation. The addition of PON1 to purified HDL extended oxidation lag phase time and reduced lipid peroxide and aldehyde accumulation by 95%, demonstrating that PON1 confers a significant antioxidant role to HDL. In addition, compared to oxidized untreated HDL, oxidized PON1-treated HDL caused a significant increase in cholesterol efflux from J-744 A.1 macrophages. This suggests that PON1's protection from HDL oxidation preserves its ability to receive cholesterol and remove it from circulation.

Animal Models.

Perhaps the most powerful evidence that PON1 protects the vasculature from atherosclerotic development comes from rodent models. Studies examining the effects of PON1 generally involve either the suppression or overexpression of the enzyme. These studies have not only identified differences in arterial plaque formation, but differences in atherosclerotic processes such as oxidative stress and adhesion molecule expression. Shih, et al. (1998) found that when fed a high-fat, high cholesterol diet, PON1 knockout (KO) mice had significantly larger aortic lesions compared to the wild-type littermates. In two studies, atherogenesis was compared between atherogenic-prone apolipoprotein E (apoE) KO mice and mice with a combined apoE KO/PON1 KO genotype (Rozenberg, Rosenblat, Coleman, Shih, & Aviram, 2003; Shih, et al., 2000). In both cases, the apoE KO/PON1 KO displayed more atherosclerosis than the apoE KO genotype alone. In addition, Rozenberg and colleagues (2003) showed that PON1 deficiency resulted in increased arterial and macrophage oxidative stress, processes that can contribute to

plaque formation. Furthermore, increases in adhesions molecules, vascular oxidative stress, and carotid thrombosis were observed by Ng, et al. (2008) in PON1-null mice fed a regular chow diet.

Conversely, mice that overexpress PON1 typically exhibit less atherosclerosis. Human PON1 transgenic (Tg) mice, which displayed a significant increase in plasma PON1 activity, had reduced atherosclerotic lesions even when high fat diet and apoE KO mouse models were examined (She, et al., 2009; Tward, et al., 2002). Plaques from the PON1 Tg/apoE KO mice also appeared to be more stable, as collagen and smooth muscle were increased and macrophage and lipid were decreased within the lesions (She, et al., 2009). Likewise, macrophage and aortic-derived oxidative stress, as well as atherosclerotic lesion size, were reduced in human PON1 Tg mice (Rozenberg, Shih, & Aviram, 2005). Overexpression of human PON1 has also been shown to reduce plaque progression in mice with combined leptin and LDL receptor deficiency, a model for metabolic syndrome (B. Mackness, Quarck, Verreth, Mackness, & Holvoet, 2006). In a study examining apoE KO, older mice (18 months) with advanced atherosclerosis, Gunset al. (2008) transfected the subjects with a PON1 adenovirus. The 13-15 fold increase in PON1 activity did not affect lesion size, but did decrease vascular oxidative stress, and improved endothelial and smooth muscle cell function. This study demonstrated that PON1 can improve vasomotor function, independent of plaque size, in older mice with preexisting atherosclerosis.

Epidemiological and Longitudinal Studies.

Several epidemiological studies have investigated the relationship between PON1 and risk, or incidence, of CVD in humans. Particularly interesting evidence emphasizing the importance of PON1 came from Navab et al.(1997), who showed that individuals with normal HDL concentration, but low PON1 activity, were more susceptible to atherosclerosis than individuals with normal HDL and significantly higher PON1 activity. Several other case-control investigations demonstrated a significant inverse relationship between CVD or its risk factors and PON1 activity and concentration (Ayub, et al., 1999; Jarvik, et al., 2000; B. Mackness, et al., 2001; McElveen, et al., 1986; Yildiz, et al., 2008), but none of these studies were able to indicate if low PON1 status was a cause or a result of CVD. This distinction can only be elucidated from longitudinal investigations.

In a recent 3-4 year longitudinal study, Bhattacharyya and colleagues (2008) assessed 1399 individuals participating in the GeneBank study who had undergone an elective diagnostic coronary angiography. This study revealed an inverse relationship between CVD prevalence and serum paraoxonase levels. Low levels of PON1 activity were significantly associated with the presence of coronary artery disease (paraoxonase: OR (odds ratio), 1.5; 95% CI, 1.03-2.3; p=.03; arylesterase: OR, 2.0; 95% CI, 1.4-3.1;p=.001]. By follow-up, the frequency of nonfatal MI or stroke among individuals within the highest quartiles of PON1 activity was only 2.5% (8/315) with paraoxonase activity and 2.8% (9/324) with arylesterase activity. The frequency of nonfatal MI or stroke within the lowest quartiles of PON1 activity was 11.9% (37/311) with paraoxonase

and 12.5% (40/319) with arylesterase activity. In addition, the researchers observed an all-cause mortality incidence of 5.4% (17/315, paraoxonase activity) and 4.9% (16/324, arylesterase activity) within the highest quartiles of PON1 activity compared to 11.9% (37/311, paraoxonase activity) and 12.9% (41/319, arylesterase activity) within the lowest quartiles. In the Caerphilly Prospective Study, 1353 men aged 49 to 65 years were monitored for coronary heart disease (CHD) events for a mean period of 15 years. The PON1 activity of the 163 participants who experienced a coronary event was 20% lower ($p= 0.039$) than the men who did not. Quintiles comparing PON1 activity to CHD risk showed an inverse, graded relationship with the median change in OR across each quintile being 0.87 (0.77 to 0.98). The results indicated that PON1 activity was an independent predictor of a new coronary event in men with preexisting CHD or at high risk for CHD. Interestingly, in this investigation there was no relationship between PON1 *concentration* and CHD (B. Mackness, et al., 2003).

Epidemiological research supports the contention that atherogenesis is not only associated with, but possibly preceded by low PON1 activity. It is also important to note that in the above epidemiological and longitudinal studies significant relationships between PON1 activity and CVD were measured using a variety of substrates including paraoxon, phenylacetate, and diazoxon. Although more research is needed to establish a causative relationship, current evidence suggests that PON1 concentration and activity may be an important factor in the prevention of atherosclerosis.

Acute Exercise Studies and Oxidized Lipid and Protein

The exercise paradox.

It is well-recognized that many health benefits result from regular aerobic physical activity. More specifically, a lifestyle void of regular physical activity merits a risk factor for cardiovascular disease (ACSM, 2006). Of the many cardiovascular benefits afforded by chronic exercise, the *reduction* of circulating ox-LDL, LDL-associated oxidized lipids, and LDL susceptibility to oxidation have been well documented (Cornelissen, Arnout, Holvoet, & Fagard, 2009; R. Elosua, et al., 2003; Rector, et al., 2007; Tomas, et al., 2002; Vasankari, Kujala, Vasankari, & Ahotupa, 1998; Ziegler, et al., 2006). With the evidence that regular physical activity acts as an antioxidant, one would expect that an isolated bout of physical activity would also serve as an antioxidant. On the contrary, an isolated bout of physical activity increases tissue utilization of oxygen and results in the propagation of free radicals (Fisher-Wellman & Bloomer, 2009). With sufficient exercise intensity and duration, the ROS overwhelm the antioxidant defenses, leading to oxidative stress. The lipid and protein components of LDL can be subjected to the increased ROS attack causing their modification (see next section). The pro-oxidant nature of acute exercise and the antioxidant nature of chronic exercise have been labeled the “exercise paradox.” This can be a point of confusion due to the beneficial effects of chronic exercise but the seemingly deleterious effects of acute exercise. Many authors have concluded that the transient increase in oxidative stress stimulates an up-regulation of several antioxidant genes (Gomez-Cabrera, Domenech, & Vina, 2008; Parthasarathy, Santanam, Ramachandran, & Meilhac, 2000). Thus exercise

training appears to lead to adaptations of the endogenous antioxidant defense system (Knez, Jenkins, & Coombes, 2007; Powers, Ji, & Leeuwenburgh, 1999), ultimately leading to a decrease in ox-LDL in the chronic exerciser. Nonetheless, acute exercise models can be used to examine the response of the antioxidant system (genes, enzymes, etc.) to a sudden increase in oxidative stress, furthering our understanding of how the antioxidant system functions and adapts to exercise.

Long duration exercise and ox-LDL.

Long endurance exercise bouts such as half-marathons, marathons, and Iron Man triathlons have resulted in elevated plasma MDA (Child, Wilkinson, Fallowfield, & Donnelly, 1998; Neubauer, Konig, Kern, Nics, & Wagner, 2008), conjugated dienes (Neubauer, et al., 2008) and decreased LDL lag phase time (Kaikkonen, et al., 2002; Liu, et al., 1999).Elevated lipid oxidation markers have been found immediately after long duration events and up to 4 days after a marathon (Liu, et al., 1999). Other long duration endurance studies have examined individuals who completed the Scientific Race of the Sant Pau Hospital, an annual run performed for the purpose of collecting medical research. This 4 hour continuous run conducted with trained individuals has produced mixed results; decreases in LDL lag phase time and increases in electronegative LDL (Benitez, et al., 2002; Sanchez-Quesada, et al., 1995; Sanchez-Quesada, et al., 1998), but no changes in plasma and LDL associated MDA (Benitez, et al., 2002) or plasma and LDL associated TBARS (Sanchez-Quesada, et al., 1998). In addition, 8 males ran 31 km with an increase in serum conjugated dienes immediately afterward, but no change in LDL conjugated dienes (Vasankari, Kujala, Vasankari, Vuorimaa, & Ahotupa, 1997).

This same investigation found no changes in serum conjugated dienes or LDL conjugated dienes after a marathon run.

Some investigations have reported *diminished* LDL associated lipid oxidation following particularly long duration exercise. Neubauer et al.(2008) found that ox-LDL decreased, but ox-LDL/LDL ratio tended to increase following an Iron Man triathlon. Vuorimaa, Ahotupa, Irjala, and Vasankari (2005) discovered decreased LDL-associated conjugated dienes after trained participants walked at 55% heart rate reserve for 6 hours. Twenty-six males and 13 females who completed the Hawaii Iron Man World Championship Triathlon showed a reduced susceptibility of plasma lipids to *in vitro* oxidation 15 minutes post-race (Ginsburg, et al., 1996). After a marathon 46 males and females showed a decrease in oxidation susceptibility of serum lipids (Kaikkonen, et al., 2002).

Unfortunately, nearly all of the long duration exercise studies failed to control for exercise intensity and dietary factors, making the results difficult to compare. Overall, it seems that aerobic exercise of relatively long duration is sufficient to induce the oxidation of circulating lipids and LDL, but not all investigators agree. More well-designed studies addressing the relationship between extended aerobic activity and oxidized lipids are warranted.

Maximal exercise tests and ox-LDL.

Ox-LDL and its associated measures in relation to maximal exercise tests are mixed, with investigations resulting in increased, decreased or no change in ox-LDL. LDL associated lipid peroxides were evaluated by Hsu, Lee, and Chen (2000) pre-, peak,

Table 2. Summary of Long Duration Exercise and Ox-LDL Studies

Citation	Exercise protocol	Lipid oxidation markers	Time points	Results
Child, et al., 1998	13.1 mile treadmill run	Plasma-MDA	Immediately pre & post	↑ immediately post
Neubauer, et al., 2008	Ironman Triathlon	Plasma-MDA, plasma-CD, ox-LDL concentration	2 days pre and immediately, 1,5, &19 days post	No change in ox-LDL, ↑ plasma-CD immediately & 1 day post, ↑ plasma-MDA 1 day post
Kaikkonen, et al., 2002	Marathon	VLDL + LDL susceptibility to oxidation, serum lipids susceptibility to oxidation	Immediately pre & post	↑ VLDL + LDL susceptibility to oxidation& ↓ serum lipids susceptibility to oxidation
Liu, et al., 1999	Marathon	LDL susceptibility to oxidation	Immediately pre and immediately & 4 days post	↑ immediately & 4 days post
Benitez, et al., 2002	4 hour run	LDL susceptibility to oxidation, LDL(-), LDL- & plasma-MDA	Immediately pre & post	↑ LDL susceptibility to oxidation and LDL(-). No change in LDL- and plasma- MDA
Sanchez-Quesada, et al., 1995	4 hour run	LDL susceptibility to oxidation, e LDL(-)	Immediately pre & post	↑ LDL susceptibility to oxidation and LDL(-)
Sanchez-Quesada, et al., 1998	4 hour run	LDL susceptibility to oxidation, LDL(-), LDL- & plasma- TBARS	Pre & between 15-30 mins post	↑ LDL susceptibility to oxidation and LDL(-). No change in LDL- & plasma-TBARS

Table 2. Summary of Long Duration Exercise and Ox-LDL Studies (Continued)

Citation	Exercise protocol	Lipid oxidation markers	Time points	Results
Vasankari, et al., 1997	31 km run and marathon	LDL- & serum-CD	Immediately pre & post	31 km run- No change in LDL-CD, ↑ serum-CD. Marathon- No change in LDL- or serum-CD
Vuorimaa, et al., 2005	6 hour walk	LDL-CD	Immediately pre & post	↓ immediately post
Ginsburg, et al., 1996	Ironman Triathlon	Plasma lipids susceptibility to oxidation	2 days pre & 15 mins post	↓ 15 mins post

Summary of Long Duration Exercise and Ox-LDL Studies. VLDL- very low density lipoprotein, LDL- low density lipoprotein, ox-LDL- oxidized low density lipoprotein, LDL(-) - electronegative LDL, MDA- malondialdehyde, CD- conjugated dienes, TBARS- thiobarbituric acid reactive substances, ↑- significant increase, ↓- significant decrease.

and 10 minutes post- a treadmill maximal exercise test. Subjects were sedentary, non-overweight/obese, and 41-59 years old. LDL lipid peroxides decreased during exercise, reaching statistical significance 10 minutes post-exercise. In another study, young non-overweight subjects cycled on a leg ergometer at approximately 50% maximal oxygen consumption for 30 minutes daily, 5 days a week, for 8 weeks, then detrained for 12 weeks. Every 4 weeks cycle ergometer max tests were performed with ox-LDL measured immediately before and after. There were no significant changes in ox-LDL after the max tests at any time point (J. S. Wang & Chow, 2004) . Immediately after a treadmill VO_2 max test congestive heart failure (CHF) patients experienced significantly elevated ox-LDL compared to baseline, while controls showed no change (Jorde, et al., 2007). On the other hand, Kostic (2009) found elevated ox-LDL concentrations in both type 2 diabetics

and age and BMI-matched control subjects following a maximal cycle ergometer test. These findings implicate, along with the CHF results from Jorde (2007), that middle aged individuals who are overweight or have a disease associated with oxidative stress (CHF, type 2 diabetes) are more likely to produce increased ox-LDL following maximal exercise. However, maximal exercise tests of relatively short duration (≤ 15 minutes) apparently will not generate elevated ox-LDL in individuals who are young or not overweight or obese. In contrast, one study found that healthy, sedentary subjects with a mean age of 25.0 years had increased susceptibility to *in vitro* LDL oxidation following a cycle ergometer max test. Unfortunately, neither BMI nor percent body fat were measured for this investigation (Tozzi-Ciancarelli, Penco, & Di Massimo, 2002). Taken together, the information concerning the response of ox-LDL to maximal exercise is not fully substantiated. Further research is required to delineate the variables involved in this dynamic.

Other acute exercise protocols and ox-LDL.

Other acute exercise studies have investigated LDL oxidation, but employed protocols other than long endurance exercise or maximal exercise tests. Eleven male professional soccer players performed treadmill exercise at 6km/hr (3.6 mi/hr) with increases of 2km/hr (1.2 mi/hr) every 3 minutes with a 1 minute rest between stages(Klapcinska, et al., 2005).The athletes performed this protocol until volitional exhaustion and displayed no significant changes in ox-LDL antibodies. Unfortunately, exercise intensity was not measured and exercise duration was not reported. However, one might infer that the exercise duration was relatively short and the exercise intensity

Table 3. Summary of Maximal Exercise Tests and Ox-LDL Studies

Citation	Exercise protocol	Lipid oxidation markers	Time points	Results
Hsu, et al., 2000	Treadmill	LDL associated lipid peroxides	Pre, peak & 10 mins post	↑ 10 mins post
J. S. Wang & Chow, 2004	Cycle ergometer	Ox-LDL concentration	Immediately pre & post	No change
Jorde, et al., 2007	Treadmill	Ox-LDL concentration	Immediately pre & post	CHF patients –↑ immediately post, controls – no change
Kostic, et al., 2007	Cycle ergometer	Ox-LDL concentration	Immediately pre & post	↑in type 2 diabetics and BMI-matched controls immediately post
Tozzi-Ciancarelli, et al., 2002	Cycle ergometer	LDL susceptibility to oxidation	Immediately pre & post	↑ immediately post

Summary of Maximal Exercise Tests and Ox-LDL Studies. LDL- low density lipoprotein, ox-LDL- oxidized low density lipoprotein, ↑- significant increase, ↓- significant decrease.

was very high, similar to a VO_2 max test. The flat results were also similar to other VO_2 max studies involving young individuals, with others showing no change in ox-LDL measures immediately after exercise (Jorde, et al., 2007; J. S. Wang & Chow, 2004).

Tozzi-Ciancarelliet al. (2002) examined LDL susceptibility to *in vitro* oxidation following 30 minutes of cycle ergometer exercise at 60% VO_2 max. Volunteers were 15 healthy, sedentary males with a mean age of 25.0 years. The investigators found no change in LDL lag time immediately post- and 24 hours post-exercise. This study reported no control of intake of antioxidants through supplement or diet. In another study,

Type 2 diabetes mellitus patients and age, gender, and BMI matched controls exercised for 40 minutes at 70-80% VO_2 peak on a cycle ergometer at the beginning and after training for 18 weeks (Iborra, et al., 2008). Ox-LDL concentration showed no difference before or after the acute bout either before or after training in patients or controls. Measurements were taken immediately before exercise as well as 40 minutes and 24 hours after the exercise session. Diet and supplement information was not measured. However, another study found a significant change in LDL susceptibility to oxidation immediately after 30 minutes of moderate intensity treadmill work. Wetzstein and colleagues (1998) exercised 12 sedentary individuals and 11 exercise trained individuals at 55% and 70% VO_2 peak, respectively, which represented a relative moderate intensity for each group. There was a similar decrease in ox-LDL lag time in both sedentary (105.3 ± 21.6 vs. 101.8 ± 23.5 min; p = NS) and trained individuals (86.0 ± 22.0 vs. 81.6 ± 18.8; p = NS), however only the total group was statistically significant (96.1 ± 23.5 vs. 92.1 ± 23.3 min; $p \leq .03$).Nonetheless, this study showed that 30 minutes of exercise has the potential to change LDL susceptibility to *in vitro* oxidation in young individuals. Subjects were not taking vitamin/mineral supplements, but diet before the exercise was not otherwise controlled. Tomás et al.(2002)measured ox-LDL via ELISA in 17 young, healthy men and women in response to acute exercise before and after 16 weeks of aerobic training. In the acute exercise sessions the subjects exercised for 30 minutes at the work rate achieved at ventilatory threshold (VT). After training, ox-LDL concentration was elevated for two hours after exercise, with the concentration peaking immediately post-exercise. Conversely, no significant change in ox-LDL occurred in

response to acute exercise *before* training. VO_{2max} and VT where reassessed at week 15 with significant increases in both fitness variables. The change in ox-LDL concentration following acute exercise could be due to the higher absolute workload achieved by the participants after training. Subjects were asked to adhere to the same diet in the 3 days before the exercise on both occasions. Additionally, no subjects were taking antioxidant supplements. Overall, research supports the contention that moderate to high intensity exercise for 30 minutes can produce a significant increase in circulating ox-LDL immediately following exercise in young healthy subjects.

Acute exercise and protein carbonyl concentration.

Protein oxidation is also a documented consequence of oxidative stress derived from acute aerobic exercise. Circulating protein carbonyl (PC) concentration is one marker commonly used to measure the degree of oxidative stress induced by physical work. Exercise studies have demonstrated that an exercise bout ranging from 30 to 120 minutes (Bloomer, Davis, Consitt, & Wideman, 2007; Bloomer, Goldfarb, & McKenzie, 2006; Goldfarb, Patrick, Bryer, & You, 2005) at intensities ranging from 70 to 80%VO_{2max} (Bloomer, et al., 2007; Bloomer, et al., 2006; Goldfarb, et al., 2005) can significantly elevate PCs. Altered PC status has been measured immediately following exercise (Alessio, et al., 2000; Bloomer, et al., 2006; Goldfarb, et al., 2005) and up to 10 hours following more than 45 minutes of exhaustive running (Michailidis, et al., 2007).

Table 4. Summary of Other Acute Exercise Protocols and Ox-LDL Studies

Citation	Exercise protocol	Lipid oxidation markers	Time points	Results
Klapcinska, et al., 2005	Progressive treadmill run with 1 min rest between stages	Ox-LDL antibodies	Pre & 3-4 mins post exercise	No change
Tozzi-Ciancarelli, et al., 2002	30 mins of cycle ergometer exercise at 60% VO_2 max	LDL susceptibility to oxidation	Immediately pre and immediately & 24 hrs post	No change
Iborra, et al., 2008	40 mins at 70-80% VO_2 peak on a cycle ergometer	Ox-LDL concentration	Immediately pre and 40 mins & 24 hrs post	No change
Wetzstein, et al., 1998	30 mins treadmill 55% (untrained) and 70% (trained) VO_{2peak}	LDL susceptibility to oxidation	Immediately pre & post	No change in individual groups, ↑ in combined group
Tomas, et al., 2002	30 mins of cycle ergometer at maximal aerobic power output	Ox-LDL concentration	Immediately pre and immediately, 30 mins, 1 hr, 2 hrs, & 24 hrs post	↑ immediately, 1 hr & 2hrs post in trained subjects

Summary of Other Acute Exercise Protocols and Ox-LDL Studies. LDL- low density lipoprotein, ox-LDL- oxidized low density lipoprotein, ↑- significant increase, ↓- significant decrease.

Exercise and PON1and Total Antioxidant Capacity

PON1 response to exercise training.

Investigations examining the PON1 activity response to aerobic exercise training have produced mixed results. The inconsistent results can be attributed to the inconsistency of the subject characteristics, exercise intervention, and possibly

measurement techniques. In essence, these studies provide no clear picture of the PON1 reaction to an exercise stimulus.

After 16 weeks of aerobic training 5 days per week for 50 minutes, Tomas et al. (2002) found no change in PON1 activity in young males and females. In a case-control study, there was no difference in PON1 levels between triathletes and controls (Brites, et al., 2006). Even with diet and exercise induced weight loss, PON1 activity and concentration decreased (Rector, et al., 2007) or was unaltered (Roberts, Ng, Hama, Eliseo, & Barnard, 2006) in overweight and obese patients. Individuals with type 2 diabetes, which is associated with low PON1 activity, produced no change in PON1 after 18 weeks of exercise training (Iborra, et al., 2008). Richter et al. (2005) investigated the effect of 12 weeks of exercise training with individuals at elevated risk or having CVD. They demonstrated that the exercise treatment had no influence on PON1 activity. Conversely, individuals with ischemic heart disease exercised for 12 weeks, which produced a 16.7% increase in PON1 activity (Goldhammer, et al., 2007). As stated previously, individuals with CVD typically associate with low PON1 activity compared to control subjects. So in this case, exercise was able to restore PON1 activity to a "normal" level. In another study, individuals with metabolic syndrome exercised at a moderate intensity for 3 months. After the training period PON1 activity increased significantly despite no observed change in HDL concentration (Casella-Filho, et al., 2011). A case control study of male and female adolescents found that adolescent basketball players had significantly higher PON1 activity (paraoxonase and arylesterase) than sedentary controls (Cakmak, Zeyrek, Atas, & Erel, 2010). Similarly, another study

investigated the PON1 activity (paraoxonase) of adolescent wrestlers (n=18) compared to sedentary controls (n=18). They found PON1 activity to be significantly higher in the wrestlers (Hamurcu, Saritas, Baskol, & Akpinar, 2010).Taken together, the research concerning the reaction of PON1 activity to aerobic exercise training is inconclusive.

PON1 response to acute exercise.

Investigations measuring PON1 activity before and after an isolated bout of exercise are scarce. Similar to exercise training results, the reaction of PON1 to acute exercise has varied. Exercise protocol, blood sample time points, measurement substrate, and subject characteristics could contribute to the inconsistent results.

Eleven marathon and ultra-marathon trained males ran a mean distance of 47.8 km (28.68 mi) in 4 hours. Blood was drawn immediately before and immediately after the run. PON1 activity was measured with no significant difference between pre and post measures (Benitez, et al., 2002). This study could indicate that a relatively long exercise bout is incapable of eliciting a PON1 response, at least in trained individuals. In another study, 10 young basketball players trained two or three times per week for 30 days. Training sessions consisted of approximately 90 minutes of basketball training. In addition, the players supplemented alpha-tocopherol during the 30-day training period. PON1 activity significantly decreased immediately after “practice” both before and after training, but alpha-tocopherol supplementation blunted the response (Tsakiris, et al., 2009). The physical activity protocol in this study was poorly controlled, leading to speculation about the exact physiological stimulus; intensity was not measured and the frequency was loosely described. Similarly, PON1 activity was assessed in adolescent

Table 5.Summary PON1 Activity Response to Chronic Exercise

Citation	Exercise protocol	Substrate	Results
Tomas, et al., 2002	16 weeks, 5 days/wk, 50 mins	Paraoxon	No change
Brites, et al., 2006	Triathletes vs. controls	Paraoxon and phenylacetate	No difference
Roberts, Ng, Hama, Eliseo, & Barnard, 2006	3 weeks, 7 days/wk, 45-60 mins, 70-85% max HR	Paraoxon	No change
Iborra, et al., 2008	18 weeks, 3 days/wk, 40 mins, 70-80% VO2 peak	Phenylacetate	No change
Rictor, et al., 2005	12 weeks, 3-5 days/wk, 30-60 mins, 60% HRR	Phenylacetate	No change
Goldhammer, et al., 2007	12 weeks, 1 day/wk, 45 mins, 70-85% HR max. 2 day/wk, 30 mins calisthenics	Phenylacetate	Increase
Casella-Filho, et al., 2011	3 months, 3 days/wk, 45 mins, moderate intensity	Paraoxon	Increase
Cakmak, Zeyrek, Atas, &Erel, 2010	adolescent basketball players vs. controls	Paraoxon and phenylacetate	Higher in athletes
Hamurcu, Saritas, Baskol, &Akpinar, 2010	Adolescent wrestlers vs. controls	Paraoxon	Higher in athletes

Summary PON1 Activity Response to Chronic Exercise. PON1- Paraoxonase 1, ↑- significant increase, ↓- significant decrease.

wrestlers before and immediately after 1.5 hours of wrestling training. The researchers found PON1 activity to be unaltered following the physical activity (Hamurcu, et al., 2010).

Type 2 diabetes mellitus patients and control subjects matched for age, gender, and BMI exercised for 40 minutes at 70-80% VO_2 peak on a cycle ergometer before and after 18 weeks of exercise training. PON1 activity showed no difference before or after the acute bout either before or after training in patients or controls. Measurements were taken immediately before exercise as well as 40 minutes and 24 hours after the exercise session (Iborra, et al., 2008). On the other hand, Tomas et al. (2002) found that PON1 activity increased after 30 minutes of intense cycling. PON1 activity was measured immediately before and after an acute bout of exercise before and after 16 weeks of supervised, aerobic training. Young, healthy volunteers cycled at their maximal aerobic power output for 30 minutes before and after the 16 weeks of training. Following training, but not before, PON1 activity was significantly higher than baseline immediately after exercise, with values returning to baseline by 30 minutes post-exercise. PON1 activity was measured before, immediately after, and two hours after a treadmill maximal exercise test in young, fit men (Otocka-Kmiecik, et al., 2010). They found a significant increase in PON1 activity immediately after the exercise with a return to baseline by 2 hours post-exercise.

To summarize, the PON1 response to an acute exercise bout has been examined in six studies. Investigations have found no effect (Benitez, et al., 2002; Hamurcu, et al., 2010; Iborra, et al., 2008), an increase (Otocka-Kmiecik, et al., 2010; Tomas, et al.,

2002), and a decrease (Tsakiris, et al., 2009) in PON1 activity following a single exercise session. Benitez et al. (2002) and Tsakiriset al. (2009) failed to report exercise intensity, making results difficult to interpret. Iborra et al.(2008) reported no change in ox-LDL or PON1 following 40 minutes of exercise at 70-80% VO_2 peak on a cycle ergometer. Unfortunately, blood was not drawn immediately following exercise, but 40 minutes and 24 hours after, possibly missing an alteration in PON1 activity. Tomás et al.(2002), as mentioned previously, reported a peak in ox-LDL immediately following 30 minutes of vigorous cycling in trained individuals. In this study elevated PON1 activity was also reported immediately after exercise. These results give rise to the specific aims of the proposed study, as they demonstrate that ox-LDL and PON1 can increase concurrently immediately following an exercise session. Unfortunately, no study has attempted to measure the *association* between alterations of ox-LDL concentration and PON1 activity after an isolated exercise bout. Furthermore, a dose-response relationship between ox-LDL and PON1 across exercise intensities (representing differing levels of oxidative stress) has not been examined.

Total antioxidant capacity.

Several enzymes, vitamins and reducing molecules comprise the complex antioxidant system within the blood. These substances work in concert to protect the body from the harmful effects of ROS. Because of the difficulty in measuring each component of the antioxidant system separately, methods have been developed to assess the total antioxidant capacity (TAC) of the serum or plasma. As a result of oxidative

Table 6. Summary PON1 Activity Response to Acute Exercise

Citation	Exercise protocol	Time points	Substrate	Results
Benitez, et al., 2002	4 hr run	Immediately pre & immediately post	Phenylacetate	No change
Tsakiris, et al., 2009	Basketball training, 90 mins	Immediately pre & immediately post, before and after training	Paraoxon and phenylacetate	Both ↓ immediately post, before and after training
Iborra, et al., 2008	40 mins at 70-80% VO_2 peak on a cycle ergometer	Immediately pre and 40 minutes & 24 hrs post	Phenylacetate	No change
Tomas, et al., 2002	30 mins at maximal aerobic power output on a cycle ergometer	Immediately pre & immediately post, before and after training	Paraoxon	↑ immediately post after 16 wks of training. No change before training
Otocka-Kmiecik, et al., 2011	Treadmill max exercise	Immediately pre, immediately post& 2 hr post	Paraoxon and phenylacetate	Both ↑ immediately post
Hamurcu, Saritas, Baskol, &Akpinar, 2010	1.5 hours of wrestling training	Immediately pre & immediately post	Paraoxon	No change

Summary PON1 Activity Response to Acute Exercise. PON1- Paraoxonase 1, ↑- significant increase, ↓- significant decrease.

stress induced by exercise, the antioxidant components of the blood are temporarily diminished in order to reduce free radicals. Thus, the detriment of TAC in response to

exercise can be used as a measure of oxidative stress. Reduced TAC has been observed following a maximal incremental exercise test (Steinberg, Delliaux, & Jammes, 2006). TAC did not change in response to 30 minutes of treadmill running at 60% VO_{2max}, but was significantly diminished immediately following nearly 40 minutes of exhaustive running (T. A. Watson, et al., 2005). There was no change in TAC immediately after 30 minutes of cycle ergometery, however TAC significantly decreased immediately following a cycle ergometer VO_{2max} test (Tozzi-Ciancarelli, et al., 2002). It appears that TAC can drop in response to relatively short, intense exercise, but intensities at 60% maximal capacity have been incapable of eliciting a change in TAC. This provides evidence that exercise intensity (level of oxidative stress) is reflected by the change in TAC immediately following exercise.

CHAPTER III

METHODS

Overview

In this study, 15 aerobically trained men (n=12) and women (n=3) aged 18-35 participated in a fitness assessment and two different exercise treatments. The fitness assessment was conducted to determine the subjects' maximal oxygen consumption ($VO_{2\ max}$). In the two subsequent exercise treatments, subjects exercised on a treadmill for 30 minutes at 60% and 80% $VO_{2\ max}$. Exercise-induced oxidative stress has been shown to be intensity dependent (Goto, et al., 2007), therefore the exercise treatments were expected to significantly alter blood concentrations of ox-LDL and PON1in a dose-dependent manner. The fitness assessment test and each of the two treatments were separated by at least one week. Treatment order was randomly counterbalanced. Blood was drawn immediately before (PRE), immediately after (0 POST), and 15 minutes after (15 POST) each exercise session. As in other studies (Tomas, et al., 2002; Wetzstein, et al., 1998), changes in ox-LDL and PON1 were expected to be at their peak immediately following exercise. However, as a safeguard, a second blood collection occurred at 15 minutes post-exercise in case the peak increase in either was delayed. Schematics detailing the timing of exercise treatments and blood collections are presented in Figures 2 and 3, respectively.

Figure 2. Timeline for Exercise Treatments

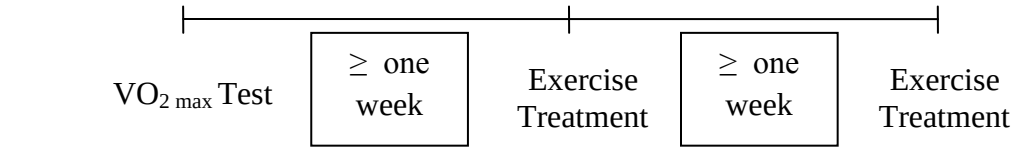
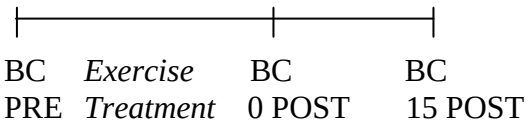


Figure 3. Timeline for Blood Collections (BC) during Each Exercise Treatment



Subject Recruitment/Selection

Exercise-trained volunteers were recruited primarily through flyers, campus publications, classroom announcements, and announcements sent to local fitness and running clubs. Exclusion criteria included history of cardiovascular disease, diabetes mellitus, dyslipidemia, physical disability, and chronic respiratory disease. Other exclusion criteria included resting blood pressure ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic), regular consumption of >2 alcoholic drinks per day, tobacco use, oral birth control use, regular vitamin or mineral supplementation within the past 2 months, and anti-inflammatory medication usage. Subjects who used anti-inflammatory treatment for acute illness were delayed by one week after discontinuation of the medication.

Individuals aged 18-35 years who performed ≥90 minutes of exercise per week at 6 or above on the 10-point Borg scale (Borg, 1998)for the past 3 months were eligible to participate in this study. Trained individuals were utilized because Tomás et al. (2002) found greater ox-LDL and PON1 responses in the trained state versus the untrained state. Participants also were required to have a body mass index (BMI) < 25 kg/m^2 or percent body fat < 20% for males or <25% for females. Criteria for participation were designed to enhance homogeneity of the cohort and control for variables that could affect lipoprotein metabolism and antioxidant status.

Fitness Assessment

Participants exercised to volitional fatigue using a horizontal treadmill (General Electric Medical Systems, Case version 6.5) running protocol. Individuals began at a pace of 120 m/min (4.5 mph) with speed increasing 10 m/min (0.3-0.4 mph) every minute until a heart rate of 120 beats per minute was achieved. From there, speed was increased 10 m/min (0.3-0.4 mph) every three minutes until volitional fatigue. Volume of oxygen consumption (VO_2) was analyzed breath-by-breath via an automated metabolic system (Parvo Medic TrueOne 2400, Sandy, UT). Criteria for achievement of maximum oxygen consumption (VO_{2max}) included the following: attainment of ≥ 95% age predicted maximal heart rate (220-age), respiratory exchange ratio (RER) of ≥ 1.15, or failure of VO_2 to increase ≥2 $mL \cdot kg^{-1} \cdot min^{-1}$ in the final minute of exercise. The three highest consecutive 20-second VO_2 values were averaged to determine VO_{2max}. During this assessment ventilatory threshold (VT) was identified as the point at which ventilation

increased disproportionally compared to the increase ofVO_2. Body fat percentage was evaluated via a seven-site skinfold test using Harpenden calipers (Creative Health Products, Ann Arbor, MI). Sex-specific equations were used to determine body fat percentage (Jackson & Pollock, 1978; Jackson, Pollock, & Ward, 1980).

Blood Collection and Analysis

Wetzstein et al.(1998) demonstrated a significant increase in LDL susceptibility to oxidation immediately following exercise. Tomas et al.(2002) measured ox-LDL concentration at several time points following exercise and found that it peaked immediately afterward. PON1 activity also peaked immediately after exercise and returned to baseline by 30 minutes post exercise. The first post-exercise PON1 measurement by Iborra et al.(2008) was at the 40 minute time point and displayed no change from baseline, possibly missing the exercise-induced change in PON1 activity. Therefore, for this investigation blood was collected PRE, 0 POST and 15 POST with each treatment session. These collection time points were chosen in an attempt to measure ox-LDL concentration and PON1 activity at their peak elevations and subsequent decline. Subjects were allowed to drink water *ad libitum* between 0 POST and 15 POST. Table 7 summarizes the blood collections and the associated blood markers.

Table 7. Blood Collections and Associated Measurement Variable

	PRE	0 POST	15 POST
Oxidized low-density lipoprotein (Ox-LDL)	X	X	X
Paraoxonase 1 (PON1)	X	X	X
Protein Carbonyl (PC)	X	X	
Total Antioxidant Capacity (TAC)	X	X	

Total cholesterol, triglyceride, LDL, and HDL concentrations were determined at baseline (the PRE time point associated with the first exercise treatment).

After a 12-hour fast, approximately 30 ml of blood was collected from an antecubital vein by a trained technician at each time point (PRE, 0 POST and 15 POST). Due to its effect on lipoprotein metabolism, participants were required to refrain from drinking alcohol for 48 hours prior to each exercise session. Blood for plasma measurements was collected into vacutainers containing EDTA and centrifuged immediately. Blood for serum measurements was collected into vacutainers with no additive and were allowed to clot at room temperature for 30 minutes before centrifugation. All samples were centrifuged in a Beckman Coulter (Allegra 6KR) centrifuge (Palo Alto, CA) at 1,500 rpm for 20 minutes at 4^{o}C. After centrifugation, plasma and serum were aliquotted and stored at -80°C.

Measurement of ox-LDL concentration.

Ox-LDL concentration was measured in plasma using a competitive enzyme-linked immunosorbent assay (ELISA) (Mercodia AB, Uppsala, Sweden).Absorbance was analyzed with a Bio-Tek microplate reader (Powerwave 340, Winooski, VT) at 450 nm. Ox-LDL was determined at PRE, 0 POST, and 15 POST. Before centrifugation small portions of whole blood were used to measure hemoglobin (Drabkin, 1948) and hematocrit (Dill & Costill, 1974). Based on these values, post-exercise ox-LDL concentrations were adjusted for plasma volume changes (Greenleaf, Convertino, & Mangseth, 1979).

Measurement of PON1 activity.

The activity of PON1 was measured in serum using the spectrophotometric OXItek Arylesterase/Paraoxonase Assay kit from the ZeptoMetrix Corporation (Buffalo, NY). This assay measures phenol formation at an absorbance of 270 nm at 25˚C, pH of 8.0. The rate of phenylacetate hydrolysis was monitored for 1 minute using a Shimadzu UV 1800 spectrophotometer (Kyoto, Japan). The activity of PON1 was determined at PRE, 0 POST, and 15 POST.

Measurement of total antioxidant capacity.

The measurement of total antioxidant capacity (TAC) was performed at the PRE and 0 POST time points associated with each exercise treatment to quantify the antioxidant environment of the blood before and after exercise. TAC was measured in serum with the Antioxidant Assay Kit from Cayman Chemicals (Ann Arbor, MI). This assay determined antioxidant capacity by measuring the ability of the serum to inhibit the

oxidation of 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate](ABTS) by metmyoglobin. The ability of the serum antioxidants to inhibit the oxidation of ABTS was compared to Trolox, a water soluble antioxidant, which provided results in mM of Trolox equivalents. The suppression of absorbance was analyzed with a Bio-Tek microplate reader (Powerwave 340, Winooski, VT) at 750 nm.

Measurement of protein carbonyl concentration.

Protein carbonyl (PC) assessment was used as a general oxidative stress marker and was also measured at time points PRE and 0 POST. PC concentration was assessed in plasma using an ELISA kit from BioCell Corporation (San Diego, CA). In this assay, absorbance was analyzed with a Bio-Tek microplate reader (Powerwave 340, Winooski, VT) at 450 nm.

Measurement of blood lipid profile.

Total cholesterol (TC), high density lipoprotein (HDL), and triglyceride (TG) concentrations were analyzed using commercialized assays from Wako Chemicals USA Inc. (Richmond, VA). Low density lipoprotein (LDL) concentration was estimated using the Friedewald formula [TC-HDL-(TG/5)] (Friedewald, Levy, & Fredrickson, 1972). Baseline LDL and HDL were assessed at the PRE time points. These measures were used for descriptive purposes and to assess if changes in ox-LDL and PON1 were related to baseline LDL and HDL concentrations, respectively.

Control of Diet and Extracurricular Physical Activity

To control for dietary influence on blood variables, 72 hour dietary food logs were collected from each participant listing all foods eaten in that timeframe before each treatment. Participants retained copies of the food logs and were instructed to consume the same diet in the 3 days prior to each exercise treatment. Participants were also instructed to refrain from consumption of alcoholic beverages for 48 hours before each blood collection.

The dietary logs were used to ensure that relevant macro- and micro-nutrient intake did not differ significantly in the days before each exercise treatment. Fat, carbohydrate, and protein intake were evaluated as well as exogenous antioxidant intake of α-tocopherol, ascorbic acid, and β-carotene (Diet Analysis Plus, Wadsworth Publishing Co., Salem, OR).

In order to prevent a detraining effect, the participants were instructed to exercise according to their regular routines in the time period between the fitness assessment and the exercise treatments. However, to prevent carry-over effects, the subjects were instructed to refrain from regular exercise 48 hours before the next laboratory exercise treatment. To assess a possible between-treatment difference in physical activity over the week leading up to each exercise treatment, the subjects completed the Kuopio Ischemic Heart Disease Study (KIHD) Seven-Day Physical Activity Recall (Lakka & Salonen, 1987) immediately before each exercise treatment.

Data Analysis

Specific aim #1: *Investigate the effect of acute exercise on ox-LDL concentration in response to two acute exercise treatments of different intensities.*

A 2 (treatment) x 3 (time) repeated measures ANOVA was utilized to assess differences in ox-LDL concentration between the treatments across the time points PRE, 0 POST, and 15 POST. Tukey's honestly significant difference test evaluated the significance of pairwise cell contrasts.

Specific aim #2: *Investigate the effect of acute exercise on PON1 activity in response to two acute exercise treatments of different intensities.*

A (treatment) x 3 (time)repeated measures ANOVA was utilized to assess differences in PON1 activity between the treatments across the time points PRE, 0 POST, and 15 POST. Tukey's honestly significant difference test evaluated the significance of pairwise cell contrasts.

Specific aim #3: *Examine the relationship between changes in ox-LDL concentration and PON1 activity in response to acute exercise.*

A Pearson product moment correlation with Bonferroni's correction for multiple comparisons was utilized to assess the relationship between changes in PON1 activity and ox-LDL concentration within each exercise treatment.

In addition, other analyses indirectly associated with the specific aims were performed. Two (treatment) by two (time) repeated measures ANOVA assessed if differences existed in both TAC and PC concentrations between and within the exercise treatments at the PRE and 0 POST time points. To assess if relationships existed between

baseline LDL and change in ox-LDL, and between baseline HDL and PON1 change, Pearson product moment correlations were performed. A repeated measures ANOVA assessed if significant differences in fat, carbohydrate, protein, α-tocopherol, ascorbic acid, and β-carotene intake existed between the exercise treatments. Finally, a paired t-test was performed to assess any differences in physical activity in the week prior to each exercise treatment. Statistical analyses were performed using the Statistical Package for the Social Sciences version 15.1 (SPSS 15.1) with statistical significance for all analyses set at $p \leq 0.05$.

Power and Sample Size Justification

This investigation employed 2 x 3 repeated measures ANOVA to assess the differences in the two exercise treatments with three blood draws associated with each treatment. A moderate effect size of 0.5 was estimated and a power level ≥ 0.80was required for statistical significance. This information was entered into G*Power (Franz Faul, Universität Kiel, Germany) which calculated that a total sample size of 14 subjects was required to meet the estimated effect size of 0.5 with a power ≥ 0.80.

CHAPTER IV

RESULTS

Participant Characteristics

Fifteen volunteers met study requirements and successfully completed all data collection procedures. Descriptive characteristics are presented in Table 8. Twelve Caucasian males and three Caucasian females participated, with significant differences between sexes existing for height, weight, body mass index, body fat percentage, fat mass, and fat-free mass. The subjects who volunteered for this study were young, fit, and displayed several healthy cardiovascular disease profiles. The mean blood lipid values fell within the healthy ranges for total cholesterol (161.0 $mg \cdot dl^{-1}$), LDL-C (91.8 $mg \cdot dl^{-1}$), HDL-C (54.6 $mg \cdot dl^{-1}$), and triglycerides (72.5 $mg \cdot dl^{-1}$) (ATP-III, 2001). In addition, BMI (22.9 $kg \cdot m^{-2}$) and body fat percentage (9.6%, males; 21.1% females) identified the subjects as free of excessive body weight and fat. The mean cardiorespiratory fitness was 56.1 $ml \cdot kg^{-1} \cdot min^{-1}$ (90^{th} percentile)and 49.5$ml \cdot kg^{-1} \cdot min^{-1}$ (90^{th} percentile) for males and females, respectively (ACSM, 2010). This elevated level of fitness was also represented in the high amount of weekly physical activity reported by the participants (7093.4 $kcal \cdot wk^{-1}$). Resting blood pressure was also healthy with average measurements of 120.5 mm Hg and 65.9 mm Hg for systolic and diastolic, respectively (Chobanian, et al., 2003). Unexpectedly, although a trend existed, VO_{2max} was not significantly higher in men than in women. There were also no significant between-sex differences in plasma lipids.

Table 8. Descriptive Characteristics

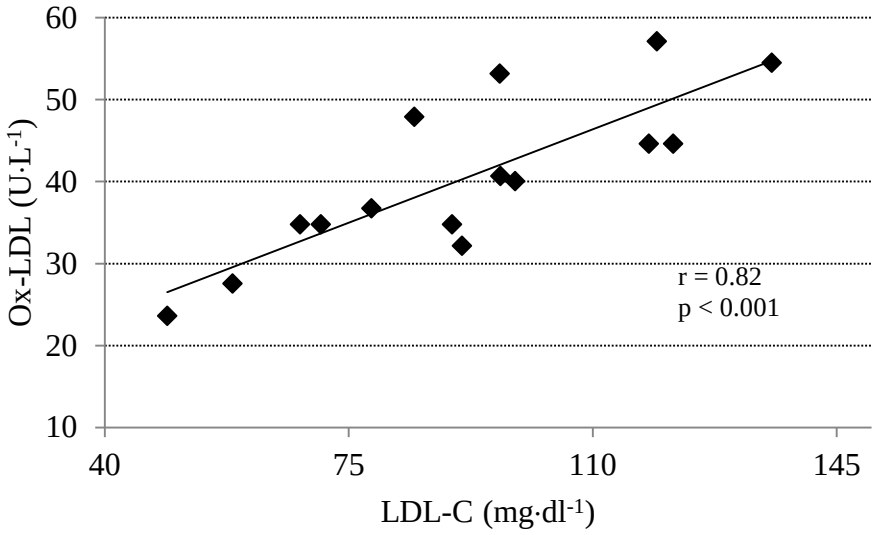
	Overall (n= 15)	Males (n=12)	Females (n=3)
Age (years)	26.3 ± 1.2	25.8 ± 1.4	28.3 ± 1.2
Height (m)	1.76 ± 0.02	1.79 ± 0.02	1.65 ± 0.02 †
Weight (kg)	71.1 ± 2.6	74.7 ± 2.0	56.4 ± 4.7 †
BMI ($kg \cdot m^{-2}$)	22.9 ± 0.5	23.4 ± 0.4	20.7 ± 1.4 *
Body fat (%)	11.9 ± 1.5	9.6 ± 0.9	21.1 ± 2.0 ‡
Fat mass (kg)	8.2 ± 0.9	7.3 ± 0.8	12.1 ± 2.2 *
Fat-free mass (kg)	62.8 ± 2.8	67.4 ± 1.7	44.3 ± 2.5 ‡
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.8 ± 1.7	56.1 ± 1.9	49.5 ± 2.5
TC ($mg \cdot dl^{-1}$)	161.0 ± 7.5	162.5 ± 7.0	154.9 ± 29.3
HDL-C ($mg \cdot dl^{-1}$)	54.6 ± 2.7	55.4 ± 3.1	51.8 ± 5.3
LDL-C ($mg \cdot dl^{-1}$)	91.8 ± 6.4	92.7 ± 6.2	88.3 ± 23.9
TG ($mg \cdot dl^{-1}$)	72.8 ± 7.3	72.5 ± 9.1	74.1 ± 5.1
$TC \cdot HDL\text{-}C^{-1}$	2.9 ± 0.1	3.0 ± 0.2	3.0 ± 0.3
Ox-LDL ($U \cdot L^{-1}$)	40.5 ± 2.5	40.8 ± 2.7	38.9 ± 8.0
PON1 activity ($kU \cdot L^{-1}$)	156.9 ± 9.3	155.5 ± 11.0	162.5 ± 19.3
Systolic BP (mm Hg)	120.5 ± 2.8	123.0 ± 3.1	110.3 ± 1.8
Diastolic BP (mm Hg)	65.9 ± 1.3	65.9 ± 1.6	66.0 ± 2.1
Leisure Time Physical Activity ($kcal \cdot wk^{-1}$)	7093.4 ± 1458.2	7812.8 ± 1774.0	4215.9 ± 73.6

Values are mean ±SEM. BMI, body mass index; TC, total cholesterol, HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglycerides; BP, blood pressure. Different across gender (*p<.05; †p<.01; ‡p<.001).

Previous research has demonstrated a significant correlation between LDL- C and ox-LDL concentration (Toshima, et al., 2000). This phenomenon was also consistent within the present study. A Pearson Product Moment correlation of 0.82 was calculated at

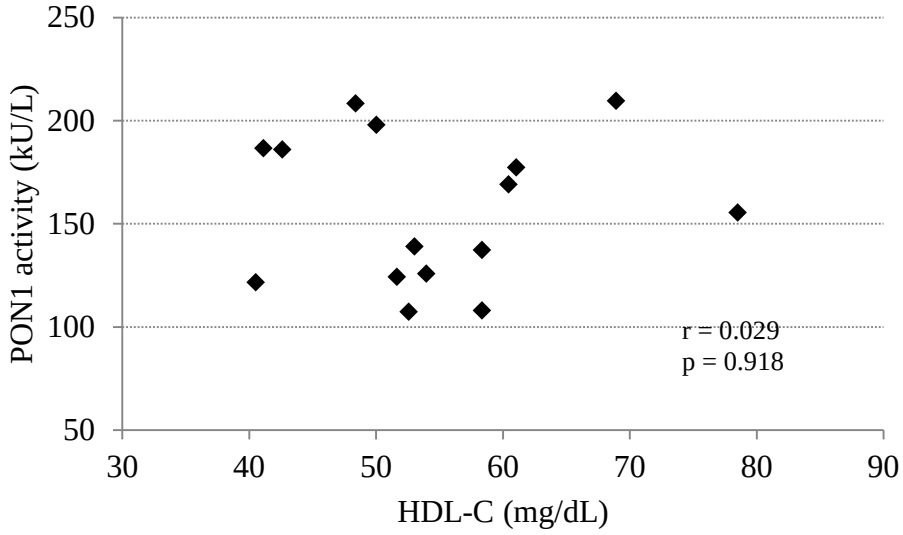
a significance of $p < 0.001$ for baseline LDL-C and baseline ox-LDL concentrations (Figure 4.)

Figure 4.Correlation between Baseline LDL-C and Baseline Ox-LDL Concentration.



Despite the fact that HDL is an important carrier for PON1, previous investigations have reported a weak or no relationship between HDL concentration and PON1 activity (Boesch-Saadatmandi, et al., 2010; Roest, et al., 2007; Tomas, et al., 2002). This was the case in the current study, as no relationship was found between the baseline concentration of HDL-C and PON1 activity ($r = 0.029$, $p = 0.918$) (Figure 5).

Figure 5.Correlation between Baseline HDL-C and Baseline PON1 Activity



Exercise Treatments

Participants performed 30 minutes of treadmill running at 60% and 80% in random order with 1 week between treatment sessions. The 60% session was performed at an average of 59.7% VO_{2max} and the 80% session was performed at 79.2%VO_{2max}. Other mean characteristics of the treatment sessions are displayed in Table 9.

Physical Activity and Diet Prior to Exercise Treatments

Paired t-tests revealed no significant differences between leisure-time physical activity and macronutrient and antioxidant vitamin intake in the days prior to either exercise treatment session (Table 10).

Table 9. Performance Data during Exercise Sessions

	60 % session	80% session	*p* value
$\%VO_{2max}$	59.7±0.6	79.2 ± 0.7	≤0.001
$VO_2(ml \cdot kg^{-1} \cdot min^{-1})$	32.7 ± 0.9	43.4 ± 1.3	≤0.001
Energy expenditure (kcal)	348.6 ± 16.7	464.7 ± 24.4	≤0.001
Kcal/kg	4.9 ± 0.1	6.5 ± 0.2	≤0.001
RER	0.88 ± 0.01	0.93 ± 0.01	≤0.001
V_E(L/min)	56.4 ± 2.7	85.9 ± 5.6	≤0.001
HR (bpm)	139.4 ± 2.2	169.6± 2.9	≤0.001
RPE (6-20 scale)	11.0 ± 0.5	14.1 ± 0.6	≤0.001

Values are mean ± SEM. RER, respiratory exchange ratio; RPE, rating of perceived exertion; V_E, minute Ventilation; HR, heart rate.

Plasma Volume Changes

Hematocrit and hemoglobin measurements were utilized to assess changes in plasma volume in response to the exercise treatments (Greenleaf, et al., 1979). Pre and post exercise hematocrit, hemoglobin and calculated plasma volume changes are presented in Table 11. Since LDL's large lipid content prevents it from freely traversing the vascular endothelium, ox-LDL values have been corrected for plasma volume shifts and are reported in the corrected state.

Table 10. Physical Activity and Diet Prior to Exercise Treatments

	3-day average prior to 60% session	3-day average prior to 80% session	*p* value
Energy intake (kcal)	2826.0±249.8	2851.5±230.5	0.916
CHO (%)	53.0±2.8	53.5 ±2.4	0.818
Fat (%)	30.4 ±2.0	31.5±1.9	0.623
PRO (%)	15.9±1.4	16.3±1.0	0.786
Cholesterol (mg)	341.4 ±52.9	384.4±70.8	0.585
Vitamin C (mg)	122.6 ±20.3	151.2±30.9	0.239
Vitamin A (μg)	1796.6 ±392.9	2214.0±446.2	0.541
Vitamin E (mg)	8.2± 2.4	7.4±1.3	0.674
	7-day total prior to 60% session	7-day total prior to 80% session	
Leisure Time Physical Activity (kcal/wk)	4289.9±750.1	4396.3±890.1	0.865

Values are mean ± SEM. CHO, carbohydrate; PRO, Protein

Table 11. Plasma Volume Correction Data

	60% session			80% session		
	PRE	0 POST	15 POST	PRE	0 POST	15 POST
Hemoglobin (g/dL)	14.5 ± 0.2	14.8 ± 0.3	14.1 ± 0.2	14.7± 0.3	15.2 ± 0.3	14.7 ± 0.3
Hematocrit (%)	44.6 ± 0.7	44.2 ± 0.8	42.9 ± 0.8	45.2 ± 0.7	45.7± 0.7	44.2 ± 0.7
Plasma Volume Δ (%)		-1.79 ± 1.2	5.35 ± 2.1		-3.96 ± 1.3	1.97 ± 1.6

Values are mean ± SEM

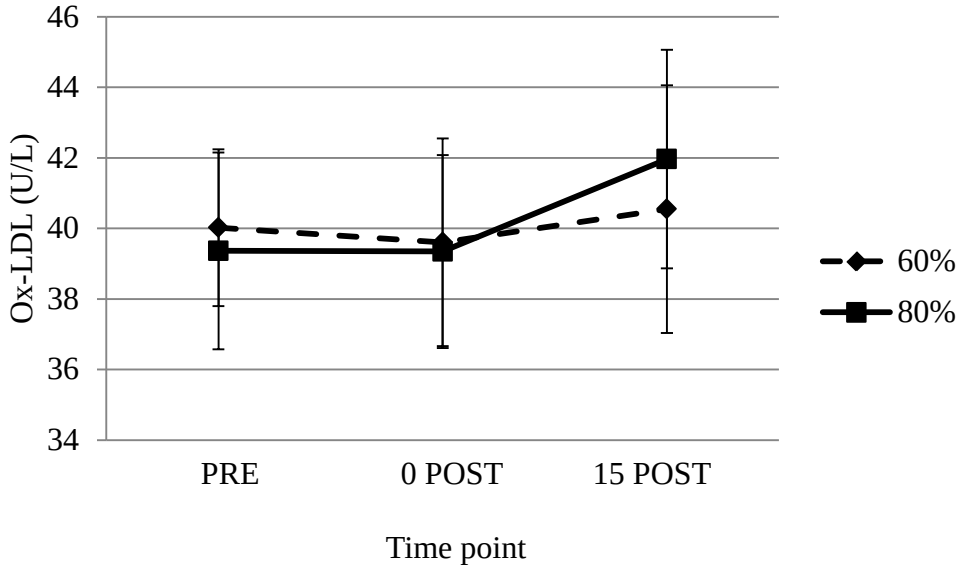
Ox-LDL and PON1 in Response to Acute Exercise

Specific Aim #1 was to "investigate the effect of acute exercise on ox-LDL concentration in response to two acute exercise treatments of different intensities."A two (treatment) by three (time point) repeated measures ANOVA was utilized to assess this aim. The between groups test indicated that the treatment variable was not significant ($F = 0.000$, $p = 0.993$), thus there was a similar response of ox-LDL concentration between the 60% exercise treatment and the 80% treatment (Figure 6). Mauchly's Test of Sphericity calculated to be significant, therefore the Huynh-Feldt F-statistic was used to assess the within variable of time. The within subjects test resulted in no significant difference in ox-LDL concentration across the time points ($F = 2.420$, $p = 0.115$), indicating that ox-LDL concentration was not significantly augmented in response to exercise. Consequently, the interaction of treatment and time point was also not significant ($F = 0.456$, $p = 0.580$). In addition, Pearson Product moment correlation found no significant relationship between VO_{2max} and changes in ox-LDL concentration at 0 POST (60%, $r = -0.018$, $p = 0.948$; 80%, $r = -0.345$, $p = 0.208$) or at 15 POST (60%, $r = 0.025$, $p = 0.930$; 80%, $r = -0.191$, $p = 0.494$). Cohen's d calculation also revealed effect sizes of 0.09 and 0.24 for the changes in ox-LDL concentration from PRE to 15 POST for the 60% and 80% treatments respectively. The effect sizes indicate a small effect of exercise on ox-LDL concentration.

Specific Aim #2 was to "investigate the effect of acute exercise on PON1 activity in response to two acute exercise treatments of different intensities." A two (treatment) by three (time point) repeated measures ANOVA was utilized to assess this aim. The

between groups test indicated that the treatment variable was not significant (F = 0.003, p = 0.960), thus there was a similar response of PON1 activity between the 60% exercise treatment and the 80% treatment (Figure 7). The within subjects test found that there was no significant difference in PON1 activity across the time points (F = 2.085, p = 0.134), indicating that PON1 activity was not significantly augmented in response to exercise. Consequently, the interaction of treatment and time point was also not significant (F = .121, p = 0.887). Cohen's d calculation also revealed effect sizes of 0.05 and 0.12 for the changes in PON1 activity from PRE to 15 POST for the 60% and 80% treatments respectively. The effect sizes indicate a small effect of exercise on PON1 activity.

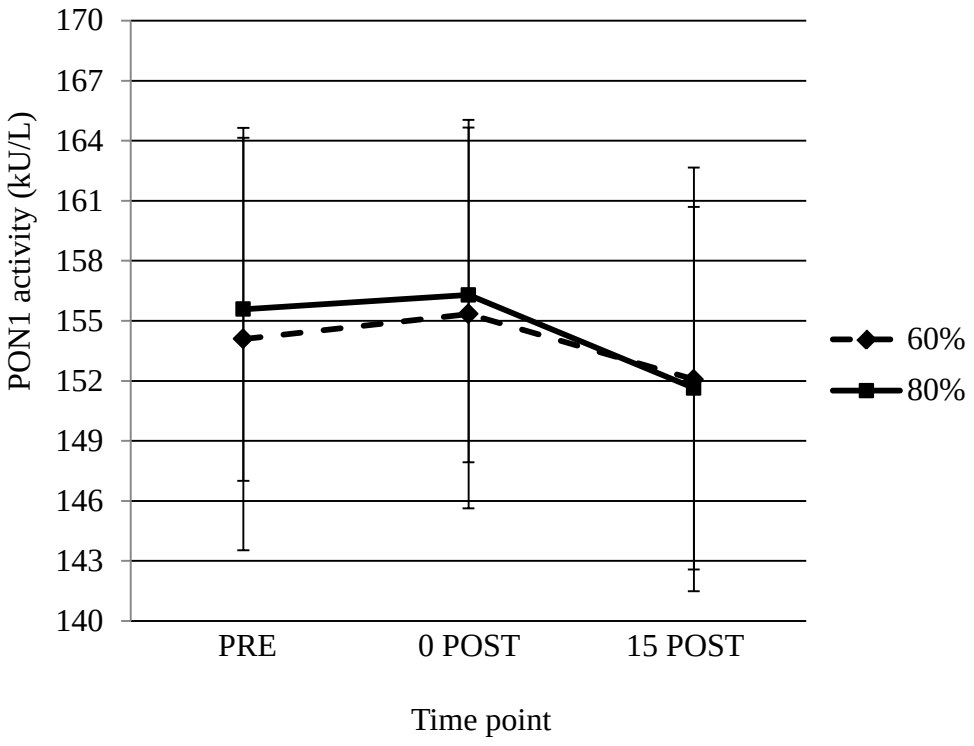
Figure 6.Ox-LDL Response to 60% and 80% Treatment Session. Bars indicate SEM.



PC measurements were analyzed PRE and 0 POST with the 60% and 80% treatment sessions (Table 5). The between groups test indicated that the treatment

variable was not significant (F = 0.168, p = 0.685), thus there was a similar response of PC between the 60% exercise treatment and the 80% treatment. The within subjects test found that there was no significant difference in PC across the time points (F = 2.053, p = 0.163), indicating that PC was not significantly augmented in response to exercise. Consequently, the interaction of treatment and time point was also not significant (F =2.253, p = 0.145).

Figure 7. PON1 Response to 60% and 80% Treatment Sessions. Bars indicate SEM.



TAC measurements were analyzed PRE and 0 POST with the 60% and 80% treatment sessions (Table 12). The between groups test indicated that the treatment variable was not significant ($F = 0.118$, $p = 0.734$), thus there was a similar response of TAC between the 60% exercise treatment and the 80% treatment. The within subjects test found that there was no significant difference in TAC across the time points ($F = 0.437$, $p = 0.514$), indicating that TAC was not significantly augmented in response to exercise. Consequently, the interaction of treatment and time point was also not significant ($F = 0.572$, $p = 0.456$).

Table 12. PC and TAC in Response to Acute Exercise

	TAC (mM of Trolox eq.)		PC (nmol/mg)	
	60% VO_{2max}	80% VO_{2max}	60% VO_{2max}	80% VO_{2max}
PRE	1.7 ± 0.1	1.7 ± 0.1	0.25 ± 0.04	0.24 ± 0.04
0 POST	1.7 ± 0.2	1.8 ± 0.2	0.28 ± 0.04	0.24 ± 0.04

Values are mean ± SEM. PC, protein carbonyls; TAC, total antioxidant capacity.

The third Aim of this study was to "examine the relationship between changes in ox-LDL concentration and PON1 activity in response to acute exercise." Pearson product moment correlation was utilized to assess this aim. The correlation between the change in ox-LDL and PON1 from PRE to 0 POST in response to 60% was 0.485 ($p = 0.067$) (Figure 8). The correlation between the change in ox-LDL and PON1 from PRE to 15 POST in response to 60% was 0.072 ($p = 0.798$) (Figure 9). The correlation between the change in ox-LDL and PON1 from PRE to 0 POST in response to 80% was -0.183 ($p = 0.513$) (Figure 10). The correlation between the change in ox-LDL and PON1 from PRE

to 15 POST in response to 80% was 0.265 ($p = 0.339$) (Figure 11). The possibility of a time lag between the change in one variable to the change in the other could exist. To assess this phenomena, correlations were conducted between the change in ox-LDL at 0 POST and the change in PON1 at 15 POST in response to 60% ($r = 0.266$, $p = 0.338$) and 80% ($r = -0.289$, $p = 0.297$). Likewise correlations were conducted between the change in PON1 at 0 POST and the change in ox-LDL at 15 POST in response to 60% ($r = 0.167$, $p = 0.551$) and 80% ($r = -0.036$, $p = 0.899$). No significant relationship was discovered between the changes in ox-LDL concentration and PON1 activity in response to either the 60% or 80% treatment sessions. Even when converted to percent change, Pearson product moment correlation revealed no significant relationship between the changes in ox-LDL concentration and PON1 activity in response to either the 60% or 80% treatment sessions. This data indicates that a change in ox-LDL was not associated with a change in PON1 activity following the exercise treatments.

Figure 8. Correlation between Ox-LDL and PON1 Changes, 60% PRE-0 POST

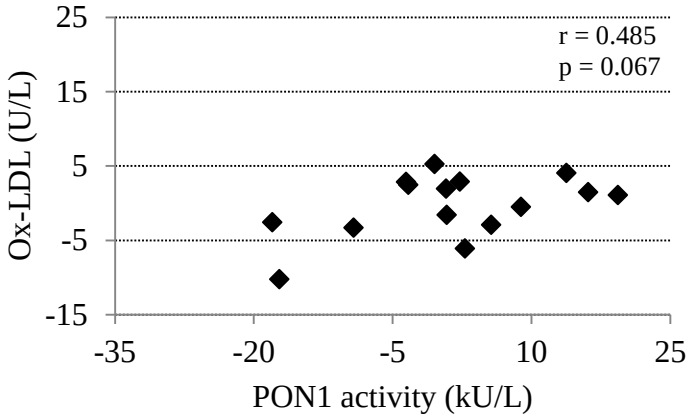


Figure 9. Correlation between Ox-LDL and PON1 Changes, 60% PRE-15 POST

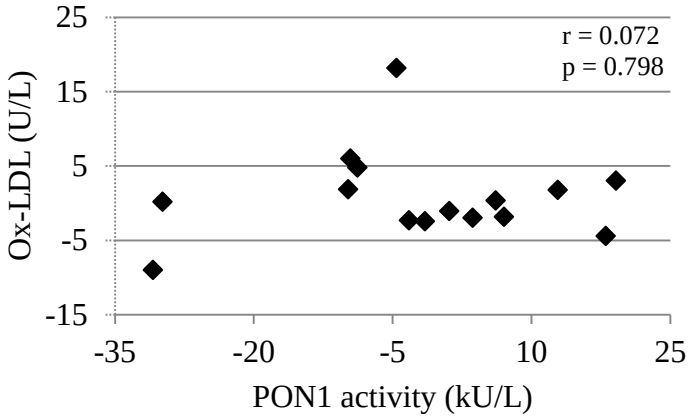


Figure 10. Correlation between Ox-LDL and PON1 Changes, 80% PRE-0 POST

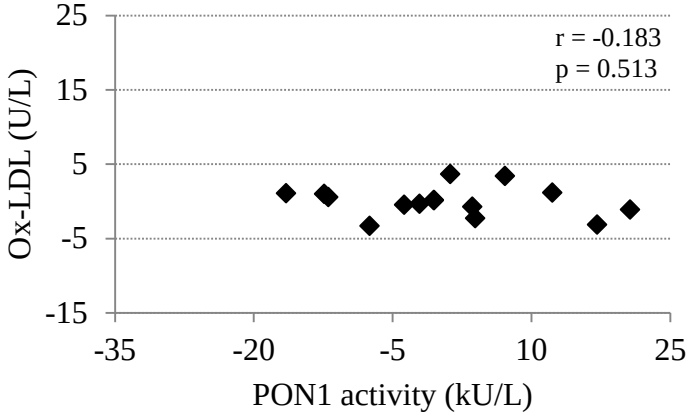
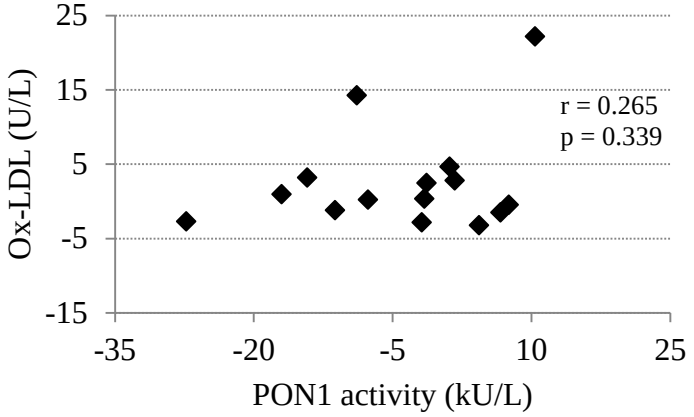


Figure 11. Correlation between Ox-LDL and PON1 Changes, 80% PRE-15 POST



Effect of Ventilatory Threshold

Performance during the 80% exercise treatment was stratified based on intensity above or below ventilatory threshold (VT). Eleven of the 15 subjects ran above their ventilatory threshold during the 80% treatment. Further analyses were performed on these 11 subjects to assess if VT moderated the variables of ox-LDL concentration, PON1 activity, PC and TAC. ANOVA revealed no significant changes in ox-LDL or PON1 across the time points PRE, 0 POST and 15 POST. Repeated measures t-tests found no significant changes in PC or TAC from PRE to 0 POST. In addition, it was speculated that magnitude of exercise intensity above VT could have an effect on the response of ox-LDL, PON1, PC and TAC. However, Pearson product moment correlations revealed no significant relationships between percent of VO_2 above VT and changes in ox-LDL concentration or PON1 activity from PRE to 15 POST or from PRE to 0 POST. According to these analyses, exercise intensity in relation to VT had no significant effect on the exercise-induced response of ox-LDL concentration, PON1 activity, PC or TAC.

Effect of Baseline Low-Density Lipoprotein and High-Density Lipoprotein

Pearson product moment correlations were used to assess the relationship between baseline LDL and HDL concentrations and changes in ox-LDL concentration and PON1 activity, respectively. No significant association was found between baseline LDL and changes in ox-LDL at 0 POST (60%, $r = 0.256$, $p = 0.356$; 80%, $r = -0.377$, $p = 0.166$) or at 15 POST (60%, $r = 0.446$, $p = 0.096$; 80%, $r = 0.082$, $p = 0.773$). Similarly, no significant relationship was found between baseline HDL and changes in PON1 activity at 0 POST (60%, $r = 0.078$, $p = 0.783$; 80%, $r = -0.051$, $p = 0.858$) or at 15 POST (60%, r

= 0.079, p = 0.778; 80%, r = -0.313, p = 0.256). Therefore, it appears that changes in ox-LDL concentration and PON1 activity were not affected by baseline concentrations of LDL and HDL, respectively.

CHAPTER V

DISCUSSION

The primary objective of this investigation was to examine the relationship between ox-LDL and PON1. To meet this objective, an acute exercise model was employed which was designed to perturb the basal homeostasis of oxidative stress, and thus ox-LDL concentration and PON1 activity. The observation of ox-LDL and PON1 in response to the exercise stimulus was intended to provide a new understanding of their association *in vivo*. To gain further knowledge of this relationship, two exercise bouts differing in intensity were utilized; each anticipated to produce different levels of oxidative stress.

Treadmill running at 60% and 80% maximal capacity for 30 minutes was chosen as the exercise protocol. Previous research has demonstrated that exercise between 70-80% $VO_{2\ max}$ is sufficient to generate elevated oxidative stress (Bryant, Ryder, Martino, Kim, & Craig, 2003; Goldfarb, McKenzie, & Bloomer, 2007; Goldfarb, et al., 2005). Therefore, the 80% treatment session was hypothesized to be an adequate stimulator of oxidative stress. Since the oxidative stress response to acute exercise has been shown to be intensity dependent (Goto, et al., 2007), the two exercise treatments were hypothesized to produce significantly different levels of oxidative stress.

Physically active individuals who participated in at least 90 minutes of exercise per week at an intensity of 6 or above on the 10 point Borg scale (Borg, 1998) for the previous 3 months were eligible to participate. Trained individuals were recruited based on the results of a previous study. Tomas et al. (2002) measured ox-LDL concentration and PON1 activity following acute exercise before and after 16 weeks of training. Following the initial acute exercise session, no change in ox-LDL concentration and PON1 activity was detected. However, after 16 weeks of exercise training the acute session was repeated with significant increases in ox-LDL concentration and PON1 activity immediately following exercise. Based on the evidence that trained subjects showed a significant change in PON1 activity in response to acute exercise but untrained subjects did not, trained subjects were recruited to participate in the current investigation.

Previous acute-model, oxidative stress studies have found significant results utilizing relatively small sample sizes. Tomas et al. (2002) recruited 17 subjects, Wetzstein et al. (1998) utilized 23 participants, while Goldfarb et al. (2005) and Bryant et al. (2003) examined 12 and 7 subjects, respectively. In the current study, a moderate effect of the treatment was anticipated, therefore and effect size of 0.5 was entered into G*Power (Franz Faul, Universität Kiel, Germany) which calculated that a total sample size of 14 subjects would be required. As a precaution, one additional subject was recruited making the total 15 subjects. In light of the previous investigations, this seemed to be a reasonable subject size to successfully power the investigation.

Male and Female Participants

Men and women were allowed to participate in this study. With that, one could speculate that hormonal difference may have compromised the results. However, previous research supports the contention that sex should not affect the outcome of this investigation. Exercise-related studies have utilized males and females when measuring ox-LDL and PON1 (Iborra, et al., 2008; Tomas, et al., 2002). Although sex differences in PON1 activity have been reported in inbred strains of mice, bin Ali et al. (2003) asserted that the genetic heterogeneity in humans should result in no sex effects in PON1 activity. Furthermore, in a human study Mueller et al. (1983) found no significant difference in serum PON1 activity between 113 males and 89 females. Also, menstrual cycle phase does not affect LDL oxidizability (Ruiz-Sanz, et al., 2007). Twenty-eight premenopausal women had blood drawn on days 3 (menstrual phase), 14 (follicular phase), and 22 (luteal phase). *In vitro* LDL oxidation, particle size, fatty acid composition, and alpha-tocopherol content were measured. The authors found no difference in oxidized LDL lag time, LDL diameter, alpha-tocopherol content, or fatty acid composition across the time points. They concluded that the menstrual cycle had no significant effect on LDL physiochemical properties or oxidizability. In addition Chung, Goldfarb, Jamurtas, and Lee (1999) found that menstrual cycle had minimal influence on oxidative stress markers in response to 30 minutes of exercise at 75-80% VO_{2max}. In the current study the effects of gender were assessed through separate analyses of the males and females. No significant results were found when analyzing males only, females only, and comparing males to females.

Ox-LDL Response to Exercise

The present study revealed no significant change in ox-LDL concentration in response to 30 minutes of aerobic treadmill exercise at 60% and 80% $VO_{2\ max}$. *Therefore, the data do not support the hypothesis that ox-LDL concentration will increase in response to acute exercise to a greater extent with the higher exercise intensity.*

Other investigations in which subjects were required to exercise for a moderate duration at a moderate to vigorous intensity have reported no change in ox-LDL or oxidized lipid markers following a single exercise session (Iborra, et al., 2008; Klapcinska, et al., 2005; Tozzi-Ciancarelli, et al., 2002). However other studies have reported elevations (Tomas, et al., 2002; Wetzstein, et al., 1998). Several issues are likely to be responsible for the disagreement amongst these and the current investigation. The most prominent of these issues are training status, exercise protocol and lipid oxidation marker.

Klapcinska et al. (2005) recruited young, fit soccer players and found an increase in ox-LDL *antibodies* following acute exercise. Not only was the ox-LDL marker different from the current investigation, but the exercise protocol involved progressive intense exercise bouts with short rest periods. Tozzi-Ciancarelli et al. (2002) found no change in LDL susceptibility to *in vitro* oxidation. However, subjects exercised at 60% $VO_{2\ max}$, an intensity that most likely was not sufficient to induce changes in ox-LDL. Iborra et al. (2008) reported no change in ox-LDL concentration following 40 minutes of cycle ergometry at 70-80% VO_2 peak in type 2 diabetes mellitus patients and controls. This study measured ox-LDL concentration with an ELISA technique (identical to the

current study) but measurements were taken 40 minutes after exercise and not immediately post, making the results difficult to compare.

Two previous studies have interesting comparisons to this dissertation. One study examined the time to *in vitro* oxidation of isolated LDL particles. This metric, although different than the one used in this dissertation, provides an indication of the susceptibility of LDL to oxidation. Wetzstein et al. (1998) found an increase in vulnerability of LDL to *in vitro* oxidation immediately following 30 minutes of treadmill exercise at 55% (untrained) and 70% (trained) VO_{2peak}. This effect was found in the total group (n=23), however when the trained (n=11) and untrained (n=12) were analyzed separately no significant increase was detected. Although possibly a sample size issue, the lack of increase in the lipid oxidation marker in the trained group is in agreement with the current study. In another investigation, Tomas et al.(2002) measured ox-LDL concentration (identical measurement as the current study) in response to 30 minutes of cycle ergometry at ventilatory threshold in individuals who had undergone 16 weeks of exercise training. They found a significant increase in ox-LDL concentration immediately, 1 hour, and 2 hours after the acute exercise session. This finding appears to be in contrast to this dissertation. However when closer comparisons are made between the fitness levels between Wetzstein et al., Tomas et al., and the current study, a noteworthy phenomenon appears. Wetzstein et al. utilized trained individuals (mean $VO_{2\ max}$ of 59.6 $ml \cdot kg^{-1} \cdot min^{-1}$) and found no change in LDL susceptibility to oxidation. This dissertation utilized trained individuals with a mean $VO_{2\ max}$ of 54.8 $ml \cdot kg^{-1} \cdot min^{-1}$ and found no change in ox-LDL concentration. Tomas et al. utilized trained individuals with

a mean $VO_{2\ max}$ of 46.2 $ml \cdot kg^{-1} \cdot min^{-1}$ and found a significant increase in ox-LDL concentration. Despite similar ox-LDL concentrations before exercise (41.1 U/L, Tomas, et al.; 39.4 U/L, current study), in the Tomas et al. study ox-LDL concentration increased approximately 8 U/L in response to exercise while, in the current study, ox-LDL increased approximately 2.5 U/L in response to the 80% treatment. The fitness level of the participants could have had an effect on the ox-LDL response to an isolated exercise bout. Perhaps individuals who are *highly* exercise trained have LDL particles that are more resistant to oxidative stress. This concept is consistent with epidemiological data that have found an inverse dose-response relationship between fitness and CVD mortality (Blair, et al., 1989).

PON1 Response to Exercise

This investigation detected no significant change in PON1 activity in response to 30 minutes of exercise at 60% and 80% $VO_{2\ max}$. *Therefore, the data do not support the hypothesis thatPON1 activity will increase in response to acute exercise to a greater extent with the higher exercise intensity.*

It appears that sufficient oxidative stress must be present in order for significant changes in PON1 activity can be detected, but this observation is not consistent across all studies. Acute exercise investigations that produced significant oxidative stress have reported mixed results. Tomas et al. (2002) found that PON1 activity increased with ox-LDL concentration immediately after exercise but then PON1 activity decreased significantly as ox-LDL remained elevated 1 and 2 hours post exercise. Tsakiris et al.

(2009) found a decrease in PON1 activity immediately after basketball practice with a concurrent decrease in total antioxidant status (an indication of oxidative stress). Thus, it appears that oxidative stress has an effect on PON1 activity, but the nature of this effect is not entirely clear. One study contrasts this, as thiobarbituric acid reactive substances(TBARS) were unchanged, yet PON1 activity increased following maximal exercise (Otocka-Kmiecik, et al., 2010). Several oxidative stress markers failed to reach significance in acute exercise studies that also found no change in PON1 activity post exercise (Benitez, et al., 2002; Hamurcu, et al., 2010; Iborra, et al., 2008). These findings are in line with the current study as PON1 activity was not significantly altered, along with oxidative stress markers (ox-LDL, PC, and TAC).

It was hypothesized in the current study that PON1 bioavailability would increase during an intense exercise bout as a mechanism to reduce exercise-induced oxidative stress, particularly the oxidative modification of LDL. However, likely due to the lack of change in the oxidative stress markers, this study provided no further insight to the PON1 activity response to an isolated exercise bout.

Through personal communication with other researchers, it is believed that the particular PC kit used in this and other studies does not provide valid results. The concentrations have been found to be unexpectedly low on several accounts.

Up-regulation of Antioxidant Protection through Chronic Exercise

Normal human serum total antioxidant capacity (TAC) contains a range of 0.5-2.0 mM of Trolox equivalents (Koracevic, Koracevic, Djordjevic, Andrejevic, & Cosic,

2001; Miller, Johnston, Collis, & Rice-Evans, 1997). Participants in this investigation had a mean TAC value before exercise of 1.7 mM of Trolox equivalents, a value that is near the upper end of the normal range. The high TAC could have protected the subjects from oxidative changes during the exercise treatments. Furthermore, TAC remained high in response to the exercise sessions. The high level of antioxidant capacity may be an effect of the habitual exercise habits of the participants.

Exercise adaptations involving the up-regulation of endogenous antioxidants are suspected to have caused high TAC and prevented oxidative stress during the 80% exercise treatment. Exercise of sufficient duration and intensity in an isolated situation has the capacity to produce reactive oxygen species (ROS) and thus, oxidative stress. The primary sources of ROS during exercise are typically from the mitochondria respiratory chain, xanthine oxidase, NADPH oxidase, and myeloperoxidase (MPO) (Bejma & Ji, 1999; Vina, et al., 2000). Increased oxygen flux during aerobic exercise can result in increased ROS generation in the mitochondria of the working muscle cells. Xanthine oxidase can product ROS when it reacts with oxygen and xanthine, a product of ATP degradation. NADPH oxidase and MPO produce ROS products through the activation of neutrophils during exercise. Because the presence of ROS is deleterious to cellular health, it has long been thought that oxidative stress is a negative consequence of physical exercise. However, now it is thought that the presence of oxidative stress is required for certain positive adaptations associated with physical activity. Indeed, oxidative stress can be viewed as having hormetic effects on the body, which is a particular dose-response relationship signifying that exposure in low doses results in positive reactions while high

doses results in negative reactions (Ji, Gomez-Cabrera, & Vina, 2006; Radak, Chung, Koltai, Taylor, & Goto, 2008). Specifically, chronic exposure to low doses of oxidative stress, as with exercise, can result in up-regulation of antioxidant defenses, ultimately resulting in a stronger antioxidant capacity of the host (Gomez-Cabrera, et al., 2008; Radak, Chung, & Goto, 2008).

Several cell signal pathways are sensitive to the presence of antioxidant and pro-oxidant substances. Hence, these cells are capable of adaptations specific to the reduction-oxidation (redox) environment to which they are subjected. One primary redox sensitive pathway of skeletal muscle involves the transcription factor nuclear factor κB (NF-κB) (Baeuerle & Baltimore, 1988).Upon activation by ROS, NF-κB has demonstrated the capacity to up-regulate gene expression of important antioxidants such as manganese superoxide dismutase (Mn-SOD), glutathione (GSH),and glutathione peroxidase (GPX) (Salminen & Vihko, 1983; Zhou, Johnson, & Rando, 2001). Mn-SOD is primarily found within the mitochondrial matrix and is the first line of defense against the mitochondrial production of ROS. Animal studies have shown that acute exercise resulted in NF-κB activation, and a subsequent increase in Mn-SOD mRNA (Gomez-Cabrera, et al., 2005; Hollander, et al., 2001). GSH and GPX are considered independent antioxidants, yet they dependent on each other to carry out their antioxidant functions. GPX catalyzes the reaction between GSH and ROS, reducing the ROS. Sekhar et al. (1997) demonstrated that the production of γ-glutamylcysteinesynthetase(GCS), the rate limiting enzyme for GSH production, is dependent on the NF-κB pathway. In addition,

oxidative stress has demonstrated the capacity to induce GPX mRNA expression via NF-κB in a mouse skeletal muscle cell culture (Zhou, et al., 2001). Furthermore, exercise training studies have found increased enzymatic activity of Mn-SOD and GPX (Roberto Elosua, et al., 2003; Higuchi, Cartier, Chen, & Holloszy, 1985; Hollander, et al., 1999; Leeuwenburgh, Fiebig, Chandwaney, & Ji, 1994; Miyazaki, et al., 2001; Powers, et al., 1994) and enhanced GSH content (Leeuwenburgh, et al., 1994; Leeuwenburgh, et al., 1997; Marin, Kretzschmar, Arokoski, Hanninen, & Klinger, 1993; Sen, Marin, Kretzschmar, & Hanninen, 1992). Thus, it appears ROS produced during exercise can act as cell signaling agents to enhance the antioxidant system.

The lack of significant change in ox-LDL, PC and TAC in the current study was most likely due to the high training status of the participants, which provided them with high antioxidant capability. A chronic stressor such as exercise can make the body more resilient to future exercise-induced oxidative stress episodes. The regular activation of the NF-κB pathway in all likelihood resulted in advanced endogenous enzymatic antioxidant capacity, particularly Mn-SOD, GPX and GSH. It seems that chronic adaptations allowed the participants to perform the more intense exercise treatment rather easily; as the average RPE was 14.1, which was below 15, or a category of “hard.” Thus, an exercise bout of 80% $VO_{2\ max}$ for 30 minutes could have been well within their antioxidant capabilities and no significant oxidative stress was observable.

Effects of Exercise above Ventilatory Threshold

Further evidence of highly developed ROS reducing capability was shown through the lack of evidence of oxidative stress in the subjects who exercised above ventilatory threshold (VT) (n=11). VT is the point at which minute ventilation increases disproportionately to oxygen consumption, and physiologically represents high aerobic energy production and increased reliance on anaerobic metabolism. The high aerobic turnout of supra-VT exercise was expected to elicit elevated ROS. However, it seems that the antioxidant ability of the participants was able to maintain homeostasis. Still this phenomenon should be interpreted with caution. When analyzing this cohort of 11 subjects the ability to reach statistical significance with this small sample size must be taken into consideration.

Relationship between Ox-LDL Concentration and PON1 Activity

No significant correlation was found at any time point between the changes in PON1 activity and ox-LDL concentration in response to the 60% and 80% exercise treatment sessions. Therefore, *the data do not support the hypothesis that a significant correlation will exist between exercise-induced changes in ox-LDL concentration and PON1 activity in response to the relatively low exercise treatment.* In addition *the data do not support the hypothesis that a significant correlation will exist between exercise-induced changes in ox-LDL concentration and PON1 activity in response to the relatively high exercise treatment is also rejected.*

The changes in ox-LDL concentration and PON1 activity are remarkably flat from PRE to 0 POST, however trends appear in both variables at 15 POST. Ox-LDL concentration average displayed a nonsignificant increase at 15 POST while PON1 activity average showed a nonsignificant decrease. These trends are slightly more prominent following the 80% treatment. This small divergence in ox-LDL concentration and PON1 activity could possibly be due to the interaction between the PON1 enzyme and the ox-LDL particles. PON1 activity has been shown to decrease upon hydrolysis of an oxidized lipid target (Aviram, et al., 1999). This phenomenon has also been attributed to the observed decrease in PON1 activity by Tomas et al. (2002) and Tsakiris et al. (2009). In the current study, the concept of an interaction between ox-LDL and PON1 and their subsequent changes is merely speculative. Unfortunately, the treatment sessions within this dissertation were insufficient to induce significant changes in ox-LDL andPON1. Therefore a conclusion concerning the *in vivo* relationship between ox-LDL and PON1 is difficult to produce.

When assessing the relationship between ox-LDL and PON1 *in vivo* one must take into consideration the method in which PON1 enzymatic activity is measured. As mentioned in Review of Literature, PON1 is considered a promiscuous enzyme due to its activity toward several substrates. At this time the only substrates for analyzing PON1 activity are substances not naturally found in the human body (paraoxon, phenylacetate, diazoxon, etc). The natural substrate for PON1 appears to be oxidized lipids. However this substrate is not used to measure PON1 activity. Research has shown that the PON1 enzyme has different methods of hydrolysis to different substrates. Aviram et al.(1998)

found that a sulfhyrdyl group was necessary for PON1 to hydrolyze oxidized lipids, but it was not required to hydrolyze paraoxon or phenylacetate. In addition, a calcium binding site seems to be very important. The chelating agent ethylenediaminetetraacetic acid (EDTA) removed calcium ions from PON1 and rendered the enzyme irreversibly inactive to phenylacetate (Kuo & La Du, 1998). However, calcium binding is not required for PON1 to protect LDL from oxidation (Aviram, Billecke, Sorenson, et al., 1998). In the current study, PON1 activity was measured in serum with no EDTA agent to retain the enzyme's calcium component. Phenylacetate was used as a substrate, so the above statements apply here; this is not the most accurate method of measuring PON1 activity *in vivo*. An oxidized lipid substrate would provide the best information regarding changes in PON1's ability to protect LDL from oxidation. This would also most likely be a more precise marker in identifying the changes in PON1 activity in relation to ox-LDL. It is possible that a change in the affinity for oxidized lipids does not mirror the change in the affinity for other substrates, like paraoxon or phenylacetate. Therefore a change in the activity toward one substrate could be altered by exercise while the activity toward another substrate reacts differently. Currently paraoxon and phenylacetate appear to be equally effective at identifying changes in PON1 activity in response to exercise, but an oxidized lipid substrate or similar substance may be needed for more accurate assessment. Future research should aim to produce an oxidized lipid-based substrate technique for the assessment of PON1 activity. Then, the nature of the *in vivo* relationship between ox-LDL concentration and PON1 activity can be more accurately assessed.

As stated in Review of Literature, the primary location of LDL oxidation is thought to be within the subendothelial space, not within the circulation. HDL can also traverse the endothelial layer where it can participate in reverse cholesterol transport by removing cholesterol from macrophages. Therefore it is believed, but not yet demonstrated, that PON1 can disrupt ox-LDL formation in the subendothelium. This is the most likely location where PON1 has the greatest capacity to act as a cardioprotective enzyme. However, this study measured the concentration of ox-LDL and activity of PON1 in the circulation. A more accurate and meaningful measurement of the *in vivo* relationship between ox-LDL and PON1 should be done in the vascular tissue. Their relationship is assumed to be very similar in the vascular tissue and the circulation, but this is not known. In addition, the effects of exercise may be different between the artery wall and the circulation. Future investigations should attempt to measure the *in vivo* dynamics of ox-LDL and PON1 in the arterial wall in animal models.

Conclusions

The antioxidant PON1 enzyme has demonstrated important cardioprotective characteristics in epidemiological and *in vitro* investigations. For these reasons, a greater understanding of how PON1 protects the vasculature could be very important. However, information regarding the nature of PON1 *in vivo* is lacking. This study attempted to ascertain new information concerning the relationship between ox-LDL and PON1. Utilizing an acute exercise model, ox-LDL concentration and PON1 activity were expected to change proportionately in response to acute exercise, signifying a biological

connection. However, no significant changes in ox-LDL concentration or PON1 activity were observed immediately and 15 minutes after treadmill running at 60% and 80% $VO_{2\ max}$. In addition, no significant correlations were measured between the changes in ox-LDL concentration and PON1 activity in response to acute exercise. Therefore, in this exercise model there was no relationship between ox-LDL and PON1. Oxidative stress in this cohort was apparently not induced through the exercise sessions, presumably due to the high training status and, as such, high antioxidant capability of the subjects. It is recommended that future investigations utilizing exercise to measure ox-LDL and PON1 recruit individuals who are not highly trained. It is still plausible that PON1 activity responds to a change in ox-LDL concentration, and more investigations are needed to elucidate the nature of this relationship.

REFERENCES

Abbott, C. A., Mackness, M., Kumar, S., Boulton, A. J. M., & Durrington, P. N. (1995). Serum paraoxonase activity, concentration, and phenotype distrubution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arteriosclerosis, Thrombosis and Vascular Biology, 15*(11), 1812-1818.

ACSM (2006). *ACSM's Guidelines for Exercise Testing and Prescription* (7th ed.). Baltimore: Lippincott, Williams & Wilkins.

ACSM (2010). *ACSM's Guildelines for Exercise Testing and Prescription* (8th ed.). Baltimore (MD): Lippincott, Williams & Wilkins.

Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., & Krieger, M. (1996). Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science, 271*(5248), 518-520.

Adkins, S., Gan, K. N., Mody, M., & La Du, B. N. (1993). Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: Glutamine or arginine at position 191, for the respective A or B allozymes. *America Journal of Human Genetics, 52*(3), 598-608.

Aldridge, W. N. (1953). An enzyme hydrolysing diethyl-p-nitrophyenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *Biochemical Journal, 53*(1), 117-124.

Alessio, H. M., Hagerman, A. E., Fulkerson, B. K., Ambrose, J., Rice, R. E., & Wiley, R. L. (2000). Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Med Sci Sports Exerc, 32*(9), 1576-1581.

ATP-III (2001). Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Journal of the American Medical Association, 285*(19), 2486-2497.

ATP-III (2002). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation, 106*(25), 3143-3421.

Aviram, M., Billecke, S., Sorensen, R., Bisgaier, C. L., Newton, R. S., Rosenblat, M., et al. (1998). Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: Selective action of human paraoxonase allozymes Q and R. *Arteriosclerosis, Thrombosis and Vascular Biology, 18*(10), 1617-1624.

Aviram, M., Billecke, S., Sorenson, R., Bisgaier, C., Newton, R., Rosenblat, M., et al. (1998). Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arterioscler Thromb Vasc Biol, 18*(10), 1617-1624.

Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N., Kaplan, M., Coleman, R., et al. (2000). Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am J Clin Nutr, 71*(5), 1062-1076.

Aviram, M., Hardak, E., Vaya, J., Mahmood, S., Milo, S., Hoffman, A., et al. (2000). Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activites. *Circulation, 101*(21), 2510-2517.

Aviram, M., & Rosenblat, M. (2004). Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. *Free Radical Biology & Medicine, 37*(9), 1307-1316.

Aviram, M., Rosenblat, M., Billecke, S., Erogul, J., Sorenson, R., Bisgaier, C. L., et al. (1999). Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radic Biol Med, 26*(7-8), 892-904.

Aviram, M., Rosenblat, M., Bisgaier, C. L., Newton, R. S., Primo-Parmo, S. L., & La Du, B. N. (1998). Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *Journal of Clinical Investigation, 101*(8), 1581-1590.

Ayub, A., Mackness, M. I., Arrol, S., Mackness, B., Patel, J., & Durrington, P. N. (1999). Serum paraoxonase after myocardial infarction. *Arterioscler Thromb Vasc Biol, 19*(2), 330-335.

Azizi, F., Raiszadeh, F., Solati, M., Etemadi, A., Rahmani, M., & Arabi, M. (2003). Serum paraoxonase 1 activity is decreased in thyroid dysfunction. *Journal of Endocrinological Investigation, 26*(8), 703-709.

Baeuerle, P. A., & Baltimore, D. (1988). Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-kappa B transcription factor. *Cell, 53*(2), 211-217.

Balogh, Z., Seres, I., Harangi, M., Kovacs, P., Kakuk, G., & Paragh, G. (2001). Gemfibrozil increases paraoxonase activity in type 2 diabetic patients. A new hypothesis of the beneficial action of fibrates? *Diabetes Metab, 27*(5 Pt 1), 604-610.

Bejma, J., & Ji, L. L. (1999). Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J Appl Physiol, 87*(1), 465-470.

Benitez, S., Sanchez-Quesada, J. L., Lucero, L., Arcelus, R., Ribas, V., Jorba, O., et al. (2002). Changes in low-density lipoprotein electronegativity and oxidizability after aerobic exercise are related to the increase in associated non-esterified fatty acids. *Atherosclerosis, 160*(1), 223-232.

Bhattacharyya, T., Nicholls, S. J., Topol, E. J., Zhang, R., Yang, X., Schmitt, D., et al. (2008). Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA, 299*(11), 1265-1276.

Biasioli, S., Schiavon, R., Petrosino, L., De Fanti, E., Cavalcanti, G., Battaglia, P., et al. (2003). Paraoxonase activity and paraoxonase 1 gene polymorphism in patients with uremia. *ASAIO J, 49*(3), 295-299.

bin Ali, A., Zhang, Q., Lim, Y. K., Fang, D., Retnam, L., & Lim, S. K. (2003). Expression of major HDL-associated antioxidant PON-1 is gender dependent and regulated during inflammation. *Free Radic Biol Med, 34*(7), 824-829.

Blair, S. N., Kohl, H. W., 3rd, Paffenbarger, R. S., Jr., Clark, D. G., Cooper, K. H., & Gibbons, L. W. (1989). Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA, 262*(17), 2395-2401.

Blatter-Garin, M. C., Kalix, B., De Pree, S., & James, R. W. (2003). Aspirin use is associated with higher serum concentrations of the anti-oxidant enzyme, paraoxonase-1. *Diabetologia, 46*(4), 593-594.

Blatter, M. C., James, R. W., Messmer, S., Barja, F., & Pometta, D. (1993). Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *Eur J Biochem, 211*(3), 871-879.

Bloomer, R. J., Davis, P. G., Consitt, L. A., & Wideman, L. (2007). Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. *Int J Sports Med, 28*(1), 21-25.

Bloomer, R. J., Goldfarb, A. H., & McKenzie, M. J. (2006). Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. *Med Sci Sports Exerc, 38*(6), 1098-1105.

Boado, R. J., Zhang, Y., Zhang, Y., Wang, Y., & Pardridge, W. M. (2008). IgG-paraoxonase-1 fusion protein for targeted drug delivery across the human blood-brain barrier. *Molecular pharmaceutics, 5*(6), 1037-1043.

Boesch-Saadatmandi, C., Rimbach, G., Schrader, C., Kofler, B. M., Armah, C. K., & Minihane, A. M. (2010). Determinants of paraoxonase activity in healthy adults. *Mol Nutr Food Res, 54*(12), 1842-1850.

Borg, G. (1998). *Borg's Perceived Exertion and Pain Scales*. Champaign, IL: Human Kinetics.

Bornscheuer, U. T., & Kazlauskas, R. J. (2004). Catalytic promiscuity in biocatalysis: using old enzymes to form new bonds and follow new pathways. *Angew Chem Int Ed Engl, 43*(45), 6032-6040.

Brites, F., Zago, V., Verona, J., Muzzio, M. L., Wikinski, R., & Schreier, L. (2006). HDL capacity to inhibit LDL oxidation in well-trained triathletes. *Life Sci, 78*(26), 3074-3081.

Bryant, R. J., Ryder, J., Martino, P., Kim, J., & Craig, B. W. (2003). Effects of vitamin E and C supplementation either alone or in combination on exercise-induced lipid peroxidation in trained cyclists. *J Strength Cond Res, 17*(4), 792-800.

Cakmak, A., Zeyrek, D., Atas, A., & Erel, O. (2010). Paraoxonase activity in athletic adolescents. *Pediatr Exerc Sci, 22*(1), 93-104.

Calabresi, L., Villa, B., Canavesi, M., Sirtori, C. R., James, R. W., Bernini, F., et al. (2004). An omega-3 polyunsaturated fatty acid concentrate increases plasma high-density lipoprotein 2 cholesterol and paraoxonase levels in patients with familial combined hyperlipidemia. *Metabolism, 53*(2), 153-158.

Camps, J., Marsillach, J., & Joven, J. (2009). The paraoxonases: role in human diseases and methodoligical difficultes in measurement. *Critical Reviews in Clinical Laboratory Science, 46*(2), 83-106.

Casella-Filho, A., Chagas, A. C., Maranhao, R. C., Trombetta, I. C., Cesena, F. H., Silva, V. M., et al. (2011). Effect of exercise training on plasma levels and functional properties of high-density lipoprotein cholesterol in the metabolic syndrome. *Am J Cardiol, 107*(8), 1168-1172.

Cathcart, M. K. (2004). Regulation of superoxide anion production by NADPH oxidase in monocytes/macrophages: contributions to atherosclerosis. *Arterioscler Thromb Vasc Biol, 24*(1), 23-28.

Child, R. B., Wilkinson, D. M., Fallowfield, J. L., & Donnelly, A. E. (1998). Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. *Med Sci Sports Exerc, 30*(11), 1603-1607.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Joseph L. Izzo, J., et al. (2003). The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: The JNC 7 Report. *Journal of the American Medical Association, 289*(19), 2560-2572.

Chung, S. C., Goldfarb, A. H., Jamurtas, A. Z., Hegde, S. S., & Lee, J. (1999). Effect of exercise during the follicular and luteal phases on indices of oxidative stress in healthy women. *Med Sci Sports Exerc, 31*(3), 409-413.

Cominacini, L., Anselmi, M., Garbin, U., Fratta Pasini, A., Stranieri, C., Fusaro, M., et al. (2005). Enhanced plasma levels of oxidized low-density lipoprotein increase circulating nuclear factor-kappa B activation in patients with unstable angina. *J Am Coll Cardiol, 46*(5), 799-806.

Copley, S. D. (2003). Enzymes with extra talents: moonlighting functions and catalytic promiscuity. *Curr Opin Chem Biol, 7*(2), 265-272.

Cornelissen, V. A., Arnout, J., Holvoet, P., & Fagard, R. H. (2009). Influence of exercise at lower and higher intensity on blood pressure and cardiovascular risk factors at older age. *J Hypertens, 27*(4), 753-762.

Costa, L. G., Cole, T. B., Jarvik, G. P., & Furlong, C. E. (2003). Functional genomics of the paraoxonase (PON1) polymorphisms: Effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. *Annual Review of Medicine, 54*, 371-392.

Costa, L. G., Richter, R. J., Murphy, S. D., & Omenn, G. S. (1987). Species differences in serum paraoxonase correlate with sensitivity to paraoxon toxicity. In L. G. Costa, C. L. Galli & S. D. Murphy (Eds.), *Toxicology of pesticides: experimental, clinical and regulatory perspectives* (pp. 263-266). Heidelberg: Springer-Verlag.

Dantoine, T. F., Debord, J., Charmes, J. P., Merle, L., Marquet, P., Lachatre, G., et al. (1998). Decrease of serum paraoxonase activity in chronic renal failure. *Journal of the American Society of Nephrology, 9*(11), 2082-2088.

Davies, H. G., Richter, R. J., Keifer, M., Broomfield, C. A., Sowalia, J., & Furlong, C. E. (1996). The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nature Genetics, 14*(3), 334-336.

Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation, 109*(23 Suppl 1), III27-32.

de Roos, N. M., Schouten, E. G., Scheek, L. M., van Tol, A., & Katan, M. B. (2002). Replacement of dietary saturated fat with trans fat reduces serum paraoxonase activity in healthy men and women. *Metabolism, 51*(12), 1534-1537.

Deakin, S. P., Leviev, I., Gomaraschi, M., Calabresi, L., Franceschini, G., & James, R. W. (2002). Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high affinity, saturable, desorption mechanism. *J Biol Chem, 277*(6), 4301-4308.

Deakin, S. P., Leviev, I., Guernier, S., & James, R. W. (2003). Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. *Arterioscler Thromb Vasc Biol, 23*(11), 2083-2089.

Dhaliwal, B. S., & Steinbrecher, U. P. (1999). Scavenger receptors and oxidized low density lipoproteins. *Clin Chim Acta, 286*(1-2), 191-205.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol, 37*(2), 247-248.

Drabkin, D. L. (1948). The standardization of hemoglobin measurement. *Am J Med Sci, 215*(1), 110.

Durrington, P. N., Mackness, B., & Mackness, M. I. (2002). The hunt for nutritional and pharmacological modulators of paraoxonase. *Arterioscler Thromb Vasc Biol, 22*(8), 1248-1250.

Durrington, P. N., Mackness, M. I., Bhatnagar, D., Julier, K., Prais, H., Arrol, S., et al. (1998). Effects of two different fibric acid derivatives on lipoproteins, cholesteryl ester transfer, fibrinogen, plasminogen activator inhibitor and paraoxonase activity in type IIb hyperlipoproteinaemia. *Atherosclerosis, 138*(1), 217-225.

Ehara, S., Ueda, M., Naruko, T., Haze, K., Itoh, A., Otsuka, M., et al. (2001). Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation, 103*(15), 1955-1960.

Elosua, R., Molina, L., Arquer, A., Sanchez-Quesada, J. L., Covas, M. I., Ordonez-Llanos, J., et al. (2003). Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis, 167*, 327-334.

Elosua, R., Molina, L., Fito, M., Arquer, A., Sanchez-Quesada, J. L., Covas, M. I., et al. (2003). Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis, 167*(2), 327-334.

Ferre, N., Camps, J., Fernandez-Ballart, J., Arija, V., Murphy, M. M., Ceruelo, S., et al. (2003). Regulation of serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population. *Clin Chem, 49*(9), 1491-1497.

Ferretti, G., Bacchetti, T., Principi, F., Di Ludovico, F., Vita, B., Angeleri, V. A., et al. (2005). Increased levels of lipid hydroperoxides in plasma of patients with multiple sclerosis: a relationship with paraoxonase activity. *Multiple Sclerosis, 11*(6), 677-682.

Fisher-Wellman, K., & Bloomer, R. J. (2009). Acute exercise and oxidative stress: a 30 year history. *Dyn Med, 8*, 1.

Florentin, M., Liberopoulos, E. N., Wierzbicki, A. S., & Mikhailidis, D. P. (2008). Multiple actions of high-density lipoprotein. *Current Opinion in Cardiology, 23*(4), 370-378.

Frei, B., Stocker, R., & Ames, B. N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci U S A, 85*(24), 9748-9752.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem, 18*(6), 499-502.

Fuhrman, B., Volkova, N., & Aviram, M. (2005). Paraoxonase 1 (PON1) is present in postprandial chylomicrons. *Atherosclerosis, 180*(1), 55-61.

Fuhrman, B., Volkova, N., & Aviram, M. (2009). Pomegranate juice polyphenols increase recombinant paraoxonase-1 binding to high-density lipoprotein: Studies in vitro and in diabetic patients. *Nutrition*.

Furlong, C. E. (2000). PON1 status and neurologic symptom complexes in Gulf War veterans. *Genome Res, 10*(2), 153-155.

Furlong, C. E., Richter, R. J., Seidel, S. L., Costa, L. G., & Motulsky, A. G. (1989). Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. *Annals of Biochemistry, 180*(2), 242-247.

Furlong, C. E., Richter, R. J., Seidel, S. L., & Motulsky, A. G. (1988). Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. *American Journal of Human Genetics, 43*(3), 230-238.

Gan, K. N., Smolen, A., Eckerson, H. W., & La Du, B. N. (1991). Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug Metab Dispos, 19*(1), 100-106.

Geldmacher-v Mallinckrodt, M., Hommel, G., & Dumbach, J. (1979). On the genetics of the human serum paraoxonase (EC 3.1.1.2). *Human Genetics, 50*(3), 313-326.

Ginsburg, G. S., Agil, A., O'Toole, M., Rimm, E., Douglas, P. S., & Rifai, N. (1996). Effects of a single bout of ultraendurance exercise on lipid levels and susceptibility of lipids to peroxidation in triathletes. *JAMA, 276*(3), 221-225.

Goldfarb, A. H., McKenzie, M. J., & Bloomer, R. J. (2007). Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation. *Appl Physiol Nutr Metab, 32*(6), 1124-1131.

Goldfarb, A. H., Patrick, S. W., Bryer, S., & You, T. (2005). Vitamin C supplementation affects oxidative-stress blood markers in response to a 30-minute run at 75% VO2max. *Int J Sport Nutr Exerc Metab, 15*(3), 279-290.

Goldhammer, E., Ben-Sira, D., Zaid, G., Biniamini, Y., Maor, I., Lanir, A., et al. (2007). Paraoxonase activity following exercise-based cardiac rehabilitation program. *J Cardiopulm Rehabil Prev, 27*(3), 151-154.

Gomez-Cabrera, M. C., Borras, C., Pallardo, F. V., Sastre, J., Ji, L. L., & Vina, J. (2005). Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol, 567*(Pt 1), 113-120.

Gomez-Cabrera, M. C., Domenech, E., & Vina, J. (2008). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic Biol Med, 44*(2), 126-131.

Goto, C., Nishioka, K., Umemura, T., Jitsuiki, D., Sakagutchi, A., Kawamura, M., et al. (2007). Acute moderate-intensity exercise induces vasodilation through an increase in nitric oxide bioavailiability in humans. *Am J Hypertens, 20*(8), 825-830.

Gouedard, C., Barouki, R., & Morel, Y. (2004a). Dietary polyphenols increase paraoxonase 1 gene expression by an aryl hydrocarbon receptor-dependent mechanism. *Mol Cell Biol, 24*(12), 5209-5222.

Gouedard, C., Barouki, R., & Morel, Y. (2004b). Induction of the paraoxonase-1 gene expression by resveratrol. *Arterioscler Thromb Vasc Biol, 24*(12), 2378-2383.

Gouedard, C., Koum-Besson, N., Barouki, R., & Morel, Y. (2003). Opposite regulation of the human paraoxonase-1 gene PON-1 by fenofibrate and statins. *Mol Pharmacol, 63*(4), 945-956.

Greenleaf, J. E., Convertino, V. A., & Mangseth, G. R. (1979). Plasma volume during stress in man: osmolality and red cell volume. *J Appl Physiol, 47*(5), 1031-1038.

Groen, A. K., Oude Elferink, R. P., Verkade, H. J., & Kuipers, F. (2004). The ins and outs of reverse cholesterol transport. *Ann Med, 36*(2), 135-145.

Guns, P.-J., Van Assche, T., Verreth, W., Fransen, P., Mackness, B., Mackness, M., et al. (2008). Paraoxonase 1 gene transfer lowers vascular oxidative stress and improves vasomotor function in apolipoprotein E-deficient mice with pre-existing atherosclerosis. *British Journal of Pharmacology, 153*(3), 508-516.

Haley, R. W., Billecke, S., & La Du, B. N. (1999). Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. *Toxicol Appl Pharmacol, 157*(3), 227-233.

Hamurcu, Z., Saritas, N., Baskol, G., & Akpinar, N. (2010). Effect of wrestling exercise on oxidative DNA damage, nitric oxide level and paraoxonase activity in adolescent boys. *Pediatr Exerc Sci, 22*(1), 60-68.

Harrison, D., Griendling, K. K., Landmesser, U., Hornig, B., & Drexler, H. (2003). Role of oxidative stress in atherosclerosis. *Am J Cardiol, 91*(3A), 7A-11A.

Havel, R. J. (1984). The formation of LDL: mechanisms and regulation. *J Lipid Res, 25*(13), 1570-1576.

Higuchi, M., Cartier, L. J., Chen, M., & Holloszy, J. O. (1985). Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. *J Gerontol, 40*(3), 281-286.

Hollander, J., Fiebig, R., Gore, M., Bejma, J., Ookawara, T., Ohno, H., et al. (1999). Superoxide dismutase gene expression in skeletal muscle: fiber-specific adaptation to endurance training. *Am J Physiol, 277*(3 Pt 2), R856-862.

Hollander, J., Fiebig, R., Gore, M., Ookawara, T., Ohno, H., & Ji, L. L. (2001). Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. *Pflugers Arch, 442*(3), 426-434.

Holvoet, P., Mertens, A., Verhamme, P., Bogaerts, K., Beyens, G., Verhaeghe, R., et al. (2001). Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol, 21*(5), 844-848.

Holvoet, P., Van Cleemput, J., Collen, D., & Vanhaecke, J. (2000). Oxidized low density lipoprotein is a prognostic marker of transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol, 20*(3), 698-702.

Hsu, H. C., Lee, Y. T., & Chen, M. F. (2000). Exercise shifts the platelet aggregation modulatory role from native to mildly oxidized low-density lipoprotein. *Med Sci Sports Exerc, 32*(5), 933-939.

Hultin, M., & Olivecrona, T. (1998). Conversion of chylomicrons into remnants. *Atherosclerosis, 141 Suppl 1*, S25-29.

Humbert, R., Adler, D. A., Disteche, C. M., Hassett, C., Omiecinski, C. J., & Furlong, C. E. (1993). The molecular basis of the human serum paraoxonase activity polymorphism. *Nature Genetics, 3*(1), 73-76.

Hussain, M. M., Fatma, S., Pan, X., & Iqbal, J. (2005). Intestinal lipoprotein assembly. *Curr Opin Lipidol, 16*(3), 281-285.

Iborra, R. T., Ribeiro, I. C., Neves, M. Q., Charf, A. M., Lottenberg, S. A., Negrao, C. E., et al. (2008). Aerobic exercise training improves the role of high-density lipoprotein antioxidant and reduces plasma lipid peroxidation in type 2 diabetes mellitus. *Scand J Med Sci Sports, 18*(6), 742-750.

Ishigaki, Y., Katagiri, H., Gao, J., Yamada, T., Imai, J., Uno, K., et al. (2008). Impact of plasma oxidized low-density lipoprotein removal on atherosclerosis. *Circulation, 118*(1), 75-83.

Ishigaki, Y., Oka, Y., & Katagiri, H. (2009). Circulating oxidized LDL: a biomarker and a pathogenic factor. *Curr Opin Lipidol, 20*(5), 363-369.

Itabe, H. (2009). Oxidative modification of LDL: its pathological role in atherosclerosis. *Clin Rev Allergy Immunol, 37*(1), 4-11.

Jackson, A. S., & Pollock, M. L. (1978). Generalized equations for predicting body density of men. *Br J Nutr, 40*(3), 497-504.

Jackson, A. S., Pollock, M. L., & Ward, A. (1980). Generalized equations for predicting body density of women. *Med Sci Sports Exerc, 12*(3), 175-181.

Jaichander, P., Selvarajan, K., Garelnabi, M., & Parthasarathy, S. (2008). Induction of paraoxonase 1 and apolipoprotein A-I gene expression by aspirin. *J Lipid Res, 49*(10), 2142-2148.

James, R. W. (2006). A long and winding road: defining the biological role and clinical importance of paraoxonases. *Clincal Chemistry and Laboratory Medicine, 44*(9), 1052-1059.

James, R. W., Blatter Garin, M. C., Calabresi, L., Miccoli, R., von Eckardstein, A., Tilly-Kiesi, M., et al. (1998). Modulated serum activities and concentrations of paraoxonase in high density lipoprotein deficiency states. *Atherosclerosis, 139*(1), 77-82.

James, R. W., Leviev, I., & Righetti, A. (2000). Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. *Circulation, 101*(19), 2252-2257.

Jarvik, G. P., Rozek, L. S., Brophy, V. H., Hatsukami, T. S., Richter, R. J., Schellenberg, G. D., et al. (2000). Paraoxonase (PON1) phenotype is a better predictor of

vascular disease than is PON1(192) or PON1(55) genotype. *Arterioscler Thromb Vasc Biol, 20*(11), 2441-2447.

Jarvik, G. P., Tsai, N. T., McKinstry, L. A., Wani, R., Brophy, V. H., Richter, R. J., et al. (2002). Vitamin C and E intake is associated with increased paraoxonase activity. *Arterioscler Thromb Vasc Biol, 22*(8), 1329-1333.

Ji, L. L., Gomez-Cabrera, M. C., & Vina, J. (2006). Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci, 1067*, 425-435.

Jorde, U. P., Colombo, P. C., Ahuja, K., Hudaihed, A., Onat, D., Diaz, T., et al. (2007). Exercise-induced increases in oxidized low-density lipoprotein are associated with adverse outcomes in chronic heart failure. *J Card Fail, 13*(9), 759-764.

Kaikkonen, J., Porkkala-Sarataho, E., Tuomainen, T. P., Nyyssonen, K., Kosonen, L., Ristonmaa, U., et al. (2002). Exhaustive exercise increases plasma/serum total oxidation resistance in moderately trained men and women, whereas their VLDL + LDL lipoprotein fraction is more susceptible to oxidation. *Scand J Clin Lab Invest, 62*(8), 599-607.

Kelso, G. J., Stuart, W. D., Richter, R. J., Furlong, C. E., Jordan-Starck, T. C., & Harmony, J. A. (1994). Apolipoprotein J is associated with paraoxonase in human plasma. *Biochemistry, 33*(3), 832-839.

Kiechl, S., Willeit, J., Mayr, M., Viehweider, B., Oberhollenzer, M., Kronenberg, F., et al. (2007). Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. *Arterioscler Thromb Vasc Biol, 27*(8), 1788-1795.

Kita, T., Kume, N., Ishii, K., Horiuchi, H., Arai, H., & Yokode, M. (1999). Oxidized LDL and expression of monocyte adhesion molecules. *Diabetes Res Clin Pract, 45*(2-3), 123-126.

Klapcinska, B., Kempa, K., Sobczak, A., Sadowska-Krepa, E., Jagsz, S., & Szoltysek, I. (2005). Evaluation of autoantibodies against oxidized LDL (oLAB) and blood antioxidant status in professional soccer players. *Int J Sports Med, 26*(1), 71-78.

Kleemola, P., Freese, R., Jauhiainen, M., Pahlman, R., Alfthan, G., & Mutanen, M. (2002). Dietary determinants of serum paraoxonase activity in healthy humans. *Atherosclerosis, 160*(2), 425-432.

Knez, W. L., Jenkins, D. G., & Coombes, J. S. (2007). Oxidative stress in half and full Ironman triathletes. *Med Sci Sports Exerc, 39*(2), 283-288.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol, 54*(5), 356-361.

Kostic, N., Caparevic, Z., Marina, D., Ilic, S., Radojkovic, J., Cosic, Z., et al. (2009). Clinical evaluation of oxidative stress in patients with diabetes mellitus type II -- impact of acute exercise. *Vojnosanit Pregl, 66*(6), 459-464.

Kume, N., Murase, T., Moriwaki, H., Aoyama, T., Sawamura, T., Masaki, T., et al. (1998). Inducible expression of lectin-like oxidized LDL receptor-1 in vascular endothelial cells. *Circ Res, 83*(3), 322-327.

Kuo, C. L., & La Du, B. N. (1998). Calcium binding by human and rabbit serum paraoxonases. Structural stability and enzymatic activity. *Drug Metab Dispos, 26*(7), 653-660.

Lakka, T. A., & Salonen, J. T. (1987). Assessment of physical activity in population studies- validity and consistency of the methods in the Kuopio Ischemic Risk Factor Study. *Scandinavian Journal of Sports Science, 9*, 89-95.

Leeuwenburgh, C., Fiebig, R., Chandwaney, R., & Ji, L. L. (1994). Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. *Am J Physiol, 267*(2 Pt 2), R439-445.

Leeuwenburgh, C., Hollander, J., Leichtweis, S., Griffiths, M., Gore, M., & Ji, L. L. (1997). Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific. *Am J Physiol, 272*(1 Pt 2), R363-369.

Leviev, I., & James, R. W. (2000). Promoter polymorphisms of the human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arteriosclerosis, Thrombosis and Vascular Biology, 20*(2), 516-521.

Li, W.-F., Costa, L. G., Richter, R. J., Hagen, T., Shih, D. M., Tward, A., et al. (2000). Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying organophosphorus compounds. *Pharmacogenetics, 10*(9), 767-779.

Libby, P. (2002). Inflammation in atherosclerosis. *Nature, 420*(6917), 868-874.

Ling, W., Lougheed, M., Suzuki, H., Buchan, A., Kodama, T., & Steinbrecher, U. P. (1997). Oxidized or acetylated low density lipoproteins are rapidly cleared by the liver in mice with disruption of the scavenger receptor class A type I/II gene. *J Clin Invest, 100*(2), 244-252.

Liu, M. L., Bergholm, R., Makimattila, S., Lahdenpera, S., Valkonen, M., Hilden, H., et al. (1999). A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. *Am J Physiol, 276*(6 Pt 1), E1083-1091.

Lopes-Virella, M. F., & Virella, G. (2010). Clinical significance of the humoral immune response to modified LDL. *Clin Immunol, 134*(1), 55-65.

Mackness, B., Davies, G. K., Turkie, W., Lee, E., Roberts, D. H., Hill, E., et al. (2001). Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? *Arterioscler Thromb Vasc Biol, 21*(9), 1451-1457.

Mackness, B., Durrington, P., McElduff, P., Yarnell, J., Azam, N., Watt, M., et al. (2003). Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. *Circulation, 107*(22), 2775-2779.

Mackness, B., Durrington, P. N., Boulton, A. J. M., Hine, D., & Mackness, M. (2002). Serum paraoxonase activity in patients with type 1 diabetes compared to healthy controls. *European Journal of Clinical Investigations, 32*(4), 259-264.

Mackness, B., Hine, D., Liu, Y., Mastorikou, M., & Mackness, M. (2004). Paraoxonase-1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells. *Biochemical and Biophysical Research Communications, 318*(3), 680-683.

Mackness, B., Mackness, M., Arrol, S., Turkie, W., & Durrington, P. N. (1998). Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Letters, 423*(1), 57-60.

Mackness, B., Mackness, M. I., Arrol, S., Turkie, W., Julier, K., Abuasha, B., et al. (1998). Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. *Atherosclerosis, 139*(2), 341-349.

Mackness, B., Quarck, R., Verreth, W., Mackness, M., & Holvoet, P. (2006). Human Paraoxonase-1 Overexpression Inhibits Atherosclerosis in a Mouse Model of Metabolic Syndrome. *Arteriosclerosis, Thrombosis and Vascular Biology, 26*(7), 1545-1550.

Mackness, M., Arrol, S., Abbott, C. A., & Durrington, P. N. (1993). Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis, 104*(1-2), 129-135.

Mackness, M., Arrol, S., & Durrington, P. N. (1991). Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Letters, 286*(1-2), 152-154.

Marin, E., Kretzschmar, M., Arokoski, J., Hanninen, O., & Klinger, W. (1993). Enzymes of glutathione synthesis in dog skeletal muscles and their response to training. *Acta Physiol Scand, 147*(4), 369-373.

Matsuura, E., Hughes, G. R., & Khamashta, M. A. (2008). Oxidation of LDL and its clinical implication. *Autoimmun Rev, 7*(7), 558-566.

McElveen, J., Mackness, M. I., Colley, C. M., Peard, T., Warner, S., & Walker, C. H. (1986). Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. *Clin Chem, 32*(4), 671-673.

Meisinger, C., Baumert, J., Khuseyinova, N., Loewel, H., & Koenig, W. (2005). Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation, 112*(5), 651-657.

Michailidis, Y., Jamurtas, A. Z., Nikolaidis, M. G., Fatouros, I. G., Koutedakis, Y., Papassotiriou, I., et al. (2007). Sampling time is crucial for measurement of aerobic exercise-induced oxidative stress. *Med Sci Sports Exerc, 39*(7), 1107-1113.

Miller, N. J., Johnston, J. D., Collis, C. S., & Rice-Evans, C. (1997). Serum total antioxidant activity after myocardial infarction. *Ann Clin Biochem, 34 (Pt 1)*, 85-90.

Miyazaki, H., Oh-ishi, S., Ookawara, T., Kizaki, T., Toshinai, K., Ha, S., et al. (2001). Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur J Appl Physiol, 84*(1-2), 1-6.

Mueller, R. F., Hornung, S., Furlong, C. E., Anderson, J., Giblett, E. R., & Motulsky, A. G. (1983). Plasma paraoxonase polymorphism: a new enzyme assay, population, family, biochemical, and linkage studies. *Am J Hum Genet, 35*(3), 393-408.

Naruko, T., Ueda, M., Ehara, S., Itoh, A., Haze, K., Shirai, N., et al. (2006). Persistent high levels of plasma oxidized low-density lipoprotein after acute myocardial infarction predict stent restenosis. *Arterioscler Thromb Vasc Biol, 26*(4), 877-883.

Navab, M., Berliner, J. A., Subbanagounder, G., Hama, S. Y., Lusis, A. J., Castellani, L. W., et al. (2001). HDL and the Inflammatory Response Induced by LDL-Derived Oxidized Phospholipids. *Arteriosclerosis, Thrombosis and Vascular Biology, 21*(4), 481-488.

Navab, M., Hama-Levy, S., Van Lenten, B. J., Fonarow, G. C., Cardinez, C. J., Castellani, L. W., et al. (1997). Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. *J Clin Invest, 99*(8), 2005-2019.

Navab, M., Hama, S. Y., Anantharamaiah, G. M., Hassan, K., Hough, G. P., Watson, A. D., et al. (2000). Normal high density lipoprotein inhibits three steps in the

formation of mildly oxidized low density lipoprotein: steps 2 and 3. *J Lipid Res, 41*(9), 1495-1508.

Navab, M., Hama, S. Y., Cooke, C. J., Anantharamaiah, G. M., Chaddha, M., Jin, L., et al. (2000). Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density liopoprotein: step 1. *Journal of Lipid Research, 41*(9), 1481-1494.

Negre-Salvayre, A., Dousset, N., Ferretti, G., Bacchetti, T., Curatola, G., & Salvayre, R. (2006). Antioxidant and cytoprotective properties of high-density lipoproteins in vascular cells. *Free Radical Biology & Medicine, 41*(7), 1031-1040.

Neubauer, O., Konig, D., Kern, N., Nics, L., & Wagner, K. H. (2008). No indications of persistent oxidative stress in response to an ironman triathlon. *Med Sci Sports Exerc, 40*(12), 2119-2128.

Ng, D. S., Chu, T., Esposito, B., Hui, P., Connelly, P. W., & Gross, P. L. (2008). Paraoxonase-1 defiiency in mice predisposes to vascular inflmmation, oxidative stress, and thrombogenicity in the absence of hyperlipidemia. *Cardiovascular Pathology, 17*(4), 226-232.

Nishi, K., Itabe, H., Uno, M., Kitazato, K. T., Horiguchi, H., Shinno, K., et al. (2002). Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler Thromb Vasc Biol, 22*(10), 1649-1654.

Nishio, E., & Watanabe, Y. (1997). Cigarette smoke extract inhibits plasma paraoxonase activity by modification of the enzyme's free thiols. *Biochem Biophys Res Commun, 236*(2), 289-293.

O'Brien, P. J., & Herschlag, D. (1999). Catalytic promiscuity and the evolution of new enzymatic activities. *Chem Biol, 6*(4), R91-R105.

Osaki, F., Ikeda, Y., Suehiro, T., Ota, K., Tsuzura, S., Arii, K., et al. (2004). Roles of Sp1 and protein kinase C in regulation of human serum paraoxonase 1 (PON1) gene transcription in HepG2 cells. *Atherosclerosis, 176*(2), 279-287.

Otocka-Kmiecik, A., Lewandowski, M., Stolarek, R., Szkudlarek, U., Nowak, D., & Orlowska-Majdak, M. (2010). Effect of single bout of maximal exercise on plasma antioxidant status and paraoxonase activity in young sportsmen. *Redox Rep, 15*(6), 275-281.

Paragh, G., Seres, I., Balogh, Z., Varga, Z., Karpati, I., Matyus, J., et al. (1998). The serum paraoxonase activity in patients with chronic renal failure and hyperlipidemia. *Nephron, 80*(2), 166-170.

Paragh, G., Seres, I., Harangi, M., Balogh, Z., Illyes, L., Boda, J., et al. (2003). The effect of micronised fenofibrate on paraoxonase activity in patients with coronary heart disease. *Diabetes Metab, 29*(6), 613-618.

Parthasarathy, S., Barnett, J., & Fong, L. G. (1990). High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta, 1044*(2), 275-283.

Parthasarathy, S., Santanam, N., Ramachandran, S., & Meilhac, O. (2000). Potential role of oxidized lipids and lipoproteins in antioxidant defense. *Free Radic Res, 33*(3), 197-215.

Pennathur, S., & Heinecke, J. W. (2007). Oxidative stress and endothelial dysfunction in vascular disease. *Current Diabetes Reports, 7*, 257-264.

Penny, W. F., Ben-Yehuda, O., Kuroe, K., Long, J., Bond, A., Bhargava, V., et al. (2001). Improvement of coronary artery endothelial dysfunction with lipid-lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. *J Am Coll Cardiol, 37*(3), 766-774.

Playfer, J. R., Eze, L. C., Bullen, M. F., & Evans, D. A. (1976). Genetic polymorphism and interethnic variability of plasma paraoxonase activity. *Journal of Medical Genetics, 13*(5), 337-342.

Podrez, E. A., Abu-Soud, H. M., & Hazen, S. L. (2000). Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic Biol Med, 28*(12), 1717-1725.

Powers, S. K., Criswell, D., Lawler, J., Ji, L. L., Martin, D., Herb, R. A., et al. (1994). Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. *Am J Physiol, 266*(2 Pt 2), R375-380.

Powers, S. K., Ji, L. L., & Leeuwenburgh, C. (1999). Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. *Med Sci Sports Exerc, 31*(7), 987-997.

Radak, Z., Chung, H. Y., & Goto, S. (2008). Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic Biol Med, 44*(2), 153-159.

Radak, Z., Chung, H. Y., Koltai, E., Taylor, A. W., & Goto, S. (2008). Exercise, oxidative stress and hormesis. *Ageing Res Rev, 7*(1), 34-42.

Rantala, M., Silaste, M. L., Tuominen, A., Kaikkonen, J., Salonen, J. T., Alfthan, G., et al. (2002). Dietary modifications and gene polymorphisms alter serum paraoxonase activity in healthy women. *J Nutr, 132*(10), 3012-3017.

Rao, M. N., Marmillot, P., Gong, M., Palmer, D. A., Seeff, L. B., Strader, D. B., et al. (2003). Light, but not heavy alcohol drinking, stimulates paraoxonase by upregulating liver mRNA in rats and humans. *Metabolism, 52*(10), 1287-1294.

Rector, R. S., Warner, S. O., Liu, Y., Hinton, P. S., Sun, G. Y., Cox, R. H., et al. (2007). Exercise and diet induced weight loss improves measures of oxidative stress and insulin sensitivity in adults with characteristics of the metabolic syndrome. *Am J Physiol Endocrinol Metab, 293*(2), E500-506.

Redgrave, T. G. (2004). Chylomicron metabolism. *Biochem Soc Trans, 32*(Pt 1), 79-82.

Richter, B., Niessner, A., Penka, M., Grdic, M., Steiner, S., Strasser, B., et al. (2005). Endurance training reduces circulating asymmetric dimethylarginine and myeloperoxidase levels in persons at risk of coronary events. *Thromb Haemost, 94*(6), 1306-1311.

Roberts, C. K., Ng, C., Hama, S., Eliseo, A. J., & Barnard, R. J. (2006). Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties

of HDL in overweight/obese men with cardiovascular risk factors. *J Appl Physiol, 101*(6), 1727-1732.

Rochu, D., Chabriere, E., & Masson, P. (2007). Human paraoxonase: a promising approach for pre-treatment and therapy of organophosphorus poisoning. *Toxicology, 233*(1-3), 47-59.

Roest, M., van Himbergen, T. M., Barendrecht, A. B., Peeters, P. H., van der Schouw, Y. T., & Voorbij, H. A. (2007). Genetic and environmental determinants of the PON-1 phenotype. *Eur J Clin Invest, 37*(3), 187-196.

Ross, R. (1993). Rous-Whipple Award Lecture. Atherosclerosis: a defense mechanism gone awry. *Am J Pathol, 143*(4), 987-1002.

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med, 340*(2), 115-126.

Rozenberg, O., Rosenblat, M., Coleman, R., Shih, D. M., & Aviram, M. (2003). Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. *Free Radical Biology & Medicine, 34*(6), 771-784.

Rozenberg, O., Shih, D. M., & Aviram, M. (2005). Paraoxonase1 (PON1) attenuates macrophage oxidative status: studies in PON1 transfected cells and in PON1 transgenic mice. *Atherosclerosis, 181*(1), 9-18.

Ruiz-Sanz, J. I., Navarro, R., Martinez, R., Hernandez, M. L., Matorras, R., & Ruiz-Larrea, M. B. (2007). No effect on menstrual cycle on LDL oxidizability and particle size. *Maturitas, 57*(3), 253-260.

Rye, K. A., & Barter, P. J. (2004). Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler Thromb Vasc Biol, 24*(3), 421-428.

Salminen, A., & Vihko, V. (1983). Lipid peroxidation in exercise myopathy. *Exp Mol Pathol, 38*(3), 380-388.

Sanchez-Quesada, J. L., Homs-Serradesanferm, R., Serrat-Serrat, J., Serra-Grima, J. R., Gonzalez-Sastre, F., & Ordonez-Llanos, J. (1995). Increase of LDL susceptibility to oxidation occurring after intense, long duration aerobic exercise. *Atherosclerosis, 118*(2), 297-305.

Sanchez-Quesada, J. L., Jorba, O., Payes, A., Otal, C., Serra-Grima, R., Gonzalez-Sastre, F., et al. (1998). Ascorbic acid inhibits the increase in low-density lipoprotein (LDL) susceptibility to oxidation and the proportion of electronegative LDL induced by intense aerobic exercise. *Coron Artery Dis, 9*(5), 249-255.

Sarandol, E., Serdar, Z., Dirican, M., & Safak, O. (2003). Effects of red wine consumption on serum paraoxonase/arylesterase activities and on lipoprotein oxidizability in healthy-men. *J Nutr Biochem, 14*(9), 507-512.

Sawamura, T., Kume, N., Aoyama, T., Moriwaki, H., Hoshikawa, H., Aiba, Y., et al. (1997). An endothelial receptor for oxidized low-density lipoprotein. *Nature, 386*(6620), 73-77.

Schneider, W. (1991). Removal of lipoprotiens form the plasma. In D. Vance & J. Vance (Eds.), *Biochemistry or lipids, lipoproteins and membranes* (pp. 261-287). Edmonton, Alberta, Canada: Elsevier Science Publishers.

Sekhar, K. R., Meredith, M. J., Kerr, L. D., Soltaninassab, S. R., Spitz, D. R., Xu, Z. Q., et al. (1997). Expression of glutathione and gamma-glutamylcysteine synthetase mRNA is Jun dependent. *Biochem Biophys Res Commun, 234*(3), 588-593.

Sen, C. K., Marin, E., Kretzschmar, M., & Hanninen, O. (1992). Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. *J Appl Physiol, 73*(4), 1265-1272.

Senti, Tomas, M., Anglada, R., Elosua, R., Marrugat, J., Covas, M. I., et al. (2003). Interrelationship of smoking, paraoxonase activity, and leisure time physical activity: a population-based study. *Eur J Intern Med, 14*(3), 178-184.

Sentí, M., Tomás, M., Fitó, M., Weinbrenner, T., Covas, M. I., Sala, J., et al. (2003). Antioxidant paraoxonase 1 activity in the metabolic syndrome. *The Journal of Clinical Endocrinology, 88*(11), 5422-5426.

Shashkin, P., Dragulev, B., & Ley, K. (2005). Macrophage differentiation to foam cells. *Curr Pharm Des, 11*(23), 3061-3072.

She, Z.-G., Zheng, W., Wei, Y.-S., Chen, H.-Z., Wang, A.-B., Li, H.-L., et al. (2009). Human paraoxonase gene cluster transgenic overexpression represses atherogenesis and promotes atherosclerotic plaque stability in apoE-null mine. *Circulation Research, 104*(10), 1160-1168.

Shelness, G. S., & Sellers, J. A. (2001). Very-low-density lipoprotein assembly and secretion. *Curr Opin Lipidol, 12*(2), 151-157.

Shih, D. M., Gu, L., Xia, Y.-R., Navab, M., Li, W.-F., Hama, S. Y., et al. (1998). Mice lacking paraoxonase are susueptible to organophosphate toxicity and atherosclerosis. *Nature, 394*(6690), 284-287.

Shih, D. M., Xia, Y.-R., Wang, X.-P., Miller, E., Castellani, L. W., Subbanagounder, G., et al. (2000). Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *The Journal of Biological Chemistry, 275*(25), 17527-17535.

Shimada, K., Mokuno, H., Matsunaga, E., Miyazaki, T., Sumiyoshi, K., Miyauchi, K., et al. (2004). Circulating oxidized low-density lipoprotein is an independent predictor for cardiac event in patients with coronary artery disease. *Atherosclerosis, 174*(2), 343-347.

Sierksma, A., van der Gaag, M. S., van Tol, A., James, R. W., & Hendriks, H. F. (2002). Kinetics of HDL cholesterol and paraoxonase activity in moderate alcohol consumers. *Alcohol Clin Exp Res, 26*(9), 1430-1435.

Smolen, A., Eckerson, H. W., Gan, K. N., Hailat, N., & La Du, B. N. (1991). Characeristics of the genetically determined allozymic forms of human serum paraoxonase/arylesterase. *Drug Metabolism and Disposition, 19*(1), 107-112.

Sorenson, R. C., Bisgaier, C. L., Aviram, M., Hsu, C., Billecke, S., & La Du, B. N. (1999). Human serum Paraoxonase/Arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids :

apolipoprotein A-I stabilizes activity. *Arterioscler Thromb Vasc Biol, 19*(9), 2214-2225.

Stary, H. C., Chandler, A. B., Dinsmore, R. E., Fuster, V., Glagov, S., Insull, W., Jr., et al. (1995). A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb Vasc Biol, 15*(9), 1512-1531.

Steinberg, J. G., Delliaux, S., & Jammes, Y. (2006). Reliability of different blood indices to explore the oxidative stress in response to maximal cycling and static exercises. *Clin Physiol Funct Imaging, 26*(2), 106-112.

Stevens, R. C., Suzuki, S. M., Cole, T. B., Park, S. S., Richter, R. J., & Furlong, C. E. (2008). Engineered recombinant human paraoxonase 1 (rHuPON1) purified from Escherichia coli protects against organophosphate poisoning. *Proc Natl Acad Sci U S A, 105*(35), 12780-12784.

Stocker, R., & Keaney, J. F., Jr. (2004). Role of oxidative modifications in atherosclerosis. *Physiol Rev, 84*(4), 1381-1478.

Suehiro, T., Nakamura, T., Inoue, M., Shiinoki, T., Ikeda, Y., Kumon, Y., et al. (2000). a polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. *Atherosclerosis, 150*(2), 295-298.

Sviridov, D., Hoang, A., Sawyer, W. H., & Fidge, N. H. (2000). Identification of a sequence of apolipoprotein A-I associated with the activation of Lecithin:Cholesterol acyltransferase. *J Biol Chem, 275*(26), 19707-19712.

Tamai, O., Matsuoka, H., Itabe, H., Wada, Y., Kohno, K., & Imaizumi, T. (1997). Single LDL apheresis improves endothelium-dependent vasodilatation in hypercholesterolemic humans. *Circulation, 95*(1), 76-82.

Tanaga, K., Bujo, H., Inoue, M., Mikami, K., Kotani, K., Takahashi, K., et al. (2002). Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arterioscler Thromb Vasc Biol, 22*(4), 662-666.

Tanimoto, N., Kumon, Y., Suehiro, T., Ohkubo, S., Ikeda, Y., Nishiya, K., et al. (2003). Serum Paraoxonase activity decrease in rheumatoid arthritis. *LIfe Sciences, 72*(25), 2877-2885.

Thomas-Moya, E., Gianotti, M., Proenza, A. M., & Llado, I. (2007). Paraoxonase 1 response to a high-fat diet: gender differences in the factors involved. *Mol Med, 13*(3-4), 203-209.

Tomas, M., Elosua, R., Sentí, M., Molina, L., Vila, J., Anglada, R., et al. (2002). Paraoxonase1-192 polymorphism modulates the effects of regular and acute exercise on paraoxonase1 activity. *Journal of Lipid Research, 43*(5), 713-720.

Tomas, M., Senti, M., Elosua, R., Vila, J., Sala, J., Masia, R., et al. (2001). Interaction between the Gln-Arg 192 variants of the paraoxonase gene and oleic acid intake as a determinant of high-density lipoprotein cholesterol and paraoxonase activity. *Eur J Pharmacol, 432*(2-3), 121-128.

Toshima, S., Hasegawa, A., Kurabayashi, M., Itabe, H., Takano, T., Sugano, J., et al. (2000). Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. *Arterioscler Thromb Vasc Biol, 20*(10), 2243-2247.

Tozzi-Ciancarelli, M. G., Penco, M., & Di Massimo, C. (2002). Influence of acute exercise on human platelet responsiveness: possible involvement of exercise-induced oxidative stress. *Eur J Appl Physiol, 86*(3), 266-272.

Tsakiris, S., Karikas, G. A., Parthimos, T., Tsakiris, T., Bakogiannis, C., & Schulpis, K. H. (2009). Alpha-tocopherol supplementation prevents the exercise-induced reduction of serum paraoxonase 1/arylesterase activities in healthy individuals. *Eur J Clin Nutr, 63*(2), 215-221.

Tsimikas, S., Bergmark, C., Beyer, R. W., Patel, R., Pattison, J., Miller, E., et al. (2003). Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J Am Coll Cardiol, 41*(3), 360-370.

Turay, J., Grniakova, V., & Valka, J. (2000). Changes in paraoxonase and apolipoprotein A-I, B, C-III and E in subjects with combined familiar hyperlipoproteinemia treated with ciprofibrate. *Drugs Exp Clin Res, 26*(3), 83-88.

Tward, A., Xia, Y.-R., Wang, X.-P., Shi, Y.-S., Park, C., Castellani, L. W., et al. (2002). Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation, 106*(4), 484-490.

Uno, M., Kitazato, K. T., Nishi, K., Itabe, H., & Nagahiro, S. (2003). Raised plasma oxidised LDL in acute cerebral infarction. *J Neurol Neurosurg Psychiatry, 74*(3), 312-316.

van der Gaag, M. S., van Tol, A., Scheek, L. M., James, R. W., Urgert, R., Schaafsma, G., et al. (1999). Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis, 147*(2), 405-410.

Vasankari, T. J., Kujala, U. M., Vasankari, T. M., & Ahotupa, M. (1998). Reduced oxidized LDL levels after a 10-month exercise program. *Med Sci Sports Exerc, 30*(10), 1496-1501.

Vasankari, T. J., Kujala, U. M., Vasankari, T. M., Vuorimaa, T., & Ahotupa, M. (1997). Effects of acute prolonged exercise on-serum and LDL oxidation and antioxidant defences. *Free Radic Biol Med, 22*(3), 509-513.

Vega-Lopez, S., Ausman, L. M., Jalbert, S. M., Erkkila, A. T., & Lichtenstein, A. H. (2006). Palm and partially hydrogenated soybean oils adversely alter lipoprotein profiles compared with soybean and canola oils in moderately hyperlipidemic subjects. *Am J Clin Nutr, 84*(1), 54-62.

Vina, J., Gimeno, A., Sastre, J., Desco, C., Asensi, M., Pallardo, F. V., et al. (2000). Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. *IUBMB Life, 49*(6), 539-544.

Vincent-Viry, M., Sass, C., Bastien, S., Aguillon, D., Siest, G., & Visvikis, S. (2003). PON1-192 phenotype and genotype assessments in 918 subjects of the Stanislas cohort study. *Clin Chem Lab Med, 41*(4), 535-540.

Vuorimaa, T., Ahotupa, M., Irjala, K., & Vasankari, T. (2005). Acute prolonged exercise reduces moderately oxidized LDL in healthy men. *Int J Sports Med, 26*(6), 420-425.

Wang, J. S., & Chow, S. E. (2004). Effects of exercise training and detraining on oxidized low-density lipoprotein-potentiated platelet function in men. *Arch Phys Med Rehabil, 85*(9), 1531-1537.

Wang, M., & Briggs, M. R. (2004). HDL: the metabolism, function, and therapeutic importance. *Chem Rev, 104*(1), 119-137.

Wang, N., & Tall, A. R. (2003). Regulation and mechanisms of ATP-binding cassette transporter A1-mediated cellular cholesterol efflux. *Arterioscler Thromb Vasc Biol, 23*(7), 1178-1184.

Watson, A. D., Berliner, J. A., Hama, S. Y., La Du, B. N., Faull, K. F., Fogelman, A. M., et al. (1995). Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest, 96*(6), 2882-2891.

Watson, T. A., Callister, R., Taylor, R. D., Sibbritt, D. W., MacDonald-Wicks, L. K., & Garg, M. L. (2005). Antioxidant restriction and oxidative stress in short-duration exhaustive exercise. *Med Sci Sports Exerc, 37*(1), 63-71.

Wetzstein, C. J., Shern-Brewer, R. A., Santanam, N., Green, N. R., White-Welkley, J. E., & Parthasarathy, S. (1998). Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? *Free Radic Biol Med, 24*(4), 679-682.

Xu, J., Kochanek, K. D., Murphy, B. S., & Tejada-Vera, B. (2010). Deaths: Final Data for 2007. *National Vital Statistics Reports, 58*(19), 1-135.

Yair, S., Ofer, B., Arik, E., Shai, S., Yossi, R., Tzvika, D., et al. (2008). Organophosphate degrading microorganisms and enzymes as biocatalysts in environmental and personal decontamination applications. *Crit Rev Biotechnol, 28*(4), 265-275.

Yildiz, A., Gur, M., Yilmaz, R., Demirbag, R., Polat, M., Selek, S., et al. (2008). Association of paraoxonase activity and coronary blood flow. *Atherosclerosis, 197*(1), 257-263.

Yla-Herttuala, S., Palinski, W., Rosenfeld, M. E., Parthasarathy, S., Carew, T. E., Butler, S., et al. (1989). Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest, 84*(4), 1086-1095.

Zechner, R. (1997). The tissue-specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism. *Curr Opin Lipidol, 8*(2), 77-88.

Zhou, L. Z., Johnson, A. P., & Rando, T. A. (2001). NF kappa B and AP-1 mediate transcriptional responses to oxidative stress in skeletal muscle cells. *Free Radic Biol Med, 31*(11), 1405-1416.

Ziegler, S., Schaller, G., Mittermayer, F., Pleiner, J., Mihaly, J., Niessner, A., et al. (2006). Exercise training improves low-density lipoprotein oxidability in untrained subjects with coronary artery disease. *Arch Phys Med Rehabil, 87*(2), 265-269.

APPENDIX A

RECRUITMENT FLYER

Test Your Fitness!

Participants are needed for a research study investigating the effect of different exercise intensities on various cardiovascular disease risk markers. Maximal oxygen consumption ($VO_{2\ max}$), body fat percentage, and cholesterol levels will also be assessed; **an approximate $200 value for free!**

You may be eligible for this study if you:

- Are between the ages of 18-35
- Exercise at least 90 minutes per week at a moderate to vigorous intensity
- Meet the study's height/weight criteria (please call or e-mail to find out if you meet this requirement)

You are not eligible for the study if you:

- Have abnormally high blood pressure (≥140 mm Hg systolic or ≥90 mm Hg diastolic)
- Use tobacco products or illegal drugs
- Drink more than an average of 2 alcoholic drinks per day
- Have diabetes or any disease affecting the heart, kidneys, or any other major organ
- Are, or think you might be, pregnant
- Currently use vitamin/mineral supplementation or hormonal birth control

Volunteers meeting the study requirements will exercise on three separate occasions in the exercise physiology laboratory. The study will last approximately two weeks. Body fat percentage, cholesterol levels, blood pressure, and $VO_{2\ max}$ will be measured throughout the study.

For more information please contact: Charlie Robison, M.S., Kinesiology Department, UNCG

573-718-7661 (cell) or cerobiso@uncg.edu

APPENDIX B

HEALTH HISTORY QUESTIONNAIRE

Health History Questionnaire

All of the information below is necessary to 1) insure your safety as a participant in this study, 2) to determine if you meet the qualification guidelines of this study, and/or 3) for the reporting of the demographics of this study. Please answer all questions accurately. If you are unsure as to how to answer any question, please notify the investigator. All information on this form will be kept confidential.

Name _____

Age _____ Birth date_____

Emergency contact: Name _____

Phone #_____

Please check the appropriate responses:

1. What is your ethnicity/race (check all that apply)?

_____African American _____White _____Asian

_____Latino _____Other (please list)_____

2. Do you use tobacco products?

_____Yes _____No

3. If you answered “no” to Question #2, have you ever used tobacco products on a regular basis in the past?

_____Yes _____No

4. If you answered “yes” to Question #3, how long ago did you quit?

_____

5. Do you regularly consume more than two alcoholic drinks per day?

_____Yes _____No

6. Have you ever been told that you have high blood pressure?

_____Yes _____No

To your best knowledge, what is your blood pressure?_____

7. Do you know your:

A) cholesterol level? _____Yes _____No If yes, what is it?_____

B) LDL-cholesterol level? _____Yes _____No If yes, what is it?_____

C) HDL-cholesterol level? _____Yes _____No If yes, what is it?_____

8. Have you ever been told by a doctor that you should not exercise or that you should only exercise under medical supervision?

_____Yes _____No

9. Have you ever had problems with chest discomfort or dizziness?

_____Yes _____No

10. Have you ever had a stroke or heart attack?

_____Yes _____No

11. Are you diabetic?

_____Yes _____No

12. Are you, or do you think that you may be, pregnant?

_____Yes _____No

13. Do you have any bleeding disorders?

_____Yes _____No

14. How many days per week do you exercise?

15. In what type(s) of exercise do you participate?

16. What is the average amount of time you spend participating in each activity?

17. Do your answers to Questions 12-15 represent your exercise habits for at least the past three months?

_____Yes _____No

18. Please list *all* prescribed and/or over-the-counter medications (including birth control), nutritional supplements, and vitamins that you take on a regular basis.

I have answered all the above questions truthfully to the best of my ability.

Signature of participant_____

Date_____

APPENDIX C

PHYSICAL ACTIVITY QUESTIONNAIRE

1. For the last twelve months, which of the following moderate or vigorous activities have you performed **regularly**? (*Please circle YES for all that apply and NO if you do not perform the activity; provide an estimate of the amount of activity for all marked YES. Be as complete as possible*).

Walking
NO YES → How many sessions per week? _____
How many miles (or fractions) per session? _____
Average duration per session? _____ (minutes)

What is your usual pace of walking? (*Please circle one*)

CASUAL or STROLLING (< 2 mph)	AVERAGE or NORMAL (2 to 3 mph)	FAIRLY BRISK (3 to 4 mph)	BRISK or STRIDING (4 mph or faster)

Stair Climbing
NO YES → How many flights of stairs do you climb UP each day? _____
(1 flight = 10 steps)

Jogging or Running
NO YES → How many sessions per week? _____
How many miles (or fractions) per session? _____
Average duration per session? _____ (minutes)

Treadmill
NO YES → How many sessions per week? _____
How many miles (or fractions) per session? _____ (minutes)
Speed?_____ (mph) Grade? _____ (%)

Bicycling
NO YES → How many sessions per week? _____
How many miles per session? _____
Average duration per session? _____ (minutes)

Swimming Laps → How many sessions per week? _____
NO YES How many miles per session?
(880 yds = 0.5 miles) _____
Average duration per session? _____ (minutes)

Aerobic Dance/Calisthenics/Floor Exercise
NO YES → How many sessions per week? _____
Average duration per session? _____ (minutes)

Moderate Sports
(e.g. Leisure volleyball, golf (not riding), social dancing, doubles tennis)
NO YES → How many sessions per week? _____
Average duration per session? _____ (minutes)

Vigorous Racquet Sports
(e.g. Racquetball, singles tennis)
NO YES → How many sessions per week? _____
Average duration per session? _____ (minutes)

Other Vigorous Sports or Exercise Involving Running
(e.g., Basketball, soccer)
NO YES → Please specify:_____
How many sessions per week? _____
Average duration per session? _____ (minutes)

Other Activities
NO YES → Please specify:_____
How many sessions per week? _____
Average duration per session? _____ (minutes)

Weight Training
(Machines, free weights)
NO YES → How many sessions per week? _____
Average duration per session? _____ (minutes)

Household Activities (i.e., Sweeping, vacuuming, washing clothes, scrubbing floors)
NO YES → How many hours per week? _____

Lawn Work and Gardening
NO YES → How many hours per week? _____

2. How many times a week do you engage in vigorous physical activity long enough to work up a sweat? _____ (*times per week*)

APPENDIX D

INFORMED CONSENT FORM

UNIVERSITY OF NORTH CAROLINA AT GREENSBORO

CONSENT TO ACT AS A HUMAN PARTICIPANT: LONG FORM

Project Title: The relationship between oxidized low-density lipoprotein and paraoxonase 1 following acute exercise

Project Director: Paul G. Davis, Ph.D.

Participant's Name: _____

What is the study about?

This is a research project.The purpose of this study is tomeasure the relationship between two substances found in the blood (oxidized low-density lipoprotein and paraoxonase-1) in response to exercise sessions of differing intensities. These substances are very important in the development of cardiovascular disease. Information obtained from this study will provide new information concerning the function of paraoxanase-1, a beneficial substance against atherosclerosis development.

Why are you asking me?

Individuals within the age of 18-35 years and who have performed vigorous aerobic exercise (running, cycling, etc.) for at least 90 minutes per week for the past 3 months are eligible to participate in this study. Reasons that you may *not* participate in this investigation include history of cardiovascular disease, diabetes mellitus, dyslipidemia, physical disability, and chronic respiratory disease. Other exclusion criteria include individuals with a body mass index (BMI) $\geq$25 kg/m^2, resting blood pressure $\geq$140 mm Hg (systolic) or $\geq$90 mm Hg (diastolic), regular consumption of >2 alcoholic drinks per day, tobacco use, oral birth control use and regular vitamin or mineral supplementation within the past 2 months.

What will you ask me to do if I agree to be in the study?

In this study, you will be asked to visit the laboratory on three different occasions: to participate in a fitness assessment test and to complete two different exercise treatments. The fitness assessment test will be used to determine your maximal oxygen consumption

($VO_{2\ max}$), a measure of cardiorespiratory fitness. This test last approximately 12 minutes and will start at a slow jogging speed and progress in intensity to where you are running up a steep incline. It is important for you to give a maximal effort during this test. However, if you feel anything unusual at any time, you should inform the researcher immediately and, if necessary, the test will be terminated. You also have the right to terminate any procedure of this experiment at any time. In the two subsequent exercise treatments, you will be asked to exercise on a treadmill for 30 minutes at 60% (moderate-to-vigorous intensity) and 80% (very vigorous intensity) $VO_{2\ max}$. You will be asked to abstain from eating or drinking any food (except water) for 12 hours before two of the exercise sessions. Blood will be drawn by a trained technician immediately before, immediately after, and 15 minutes after these two exercise sessions. You will also be asked to record food intake and recall physical activity prior to two of the exercise sessions. The maximal exercise session will last approximately 45 minutes. Exercise treatment sessions will last approximately 75 minutes. The entire study lasts approximately 2 weeks. Participants may call or email Charles Robison (573-718-7661, cerobiso@uncg.edu) or Paul Davis (336-334-3030, pgdavis@uncg.edu) with any questions about these procedures.

What are the dangers to me?

Maximal exercise testing and moderate/high-intensity exercise carry a very remote chance of a heart attack or sudden death. Fit, young individuals (ages 18-35) rarely experience cardiac events. Research has found that exercise related death among young athletes is one per 133,000 men and 1 per 769,000 women, with the most common causes of death stemming from congenital or hereditary cardiac abnormalities.Safety shall be enhanced through use of trained personnel in blood collection procedures and administration of maximal graded exercise test. Standard emergency protocol will include, if needed, cardiopulmonary resuscitation (CPR), use of an automated external defibrillator, and notification of the Emergency Medical Service (EMS). In addition, there is a slight possibility that you will feel weak or faint from fasting. You should tell the experimenter immediately if you experience any unusual sensations before, during, or after exercise. Since you do not have known heart, lung, or metabolic disease, do not smoke, and do not have severely high blood pressure, the potential benefit of participation most likely outweighs the risk. In addition, a chance of bruising and a very slight chance of infection exists with blood collection. This will be minimized through use of a trained technician and sterile materials. Neither the investigator nor UNCG will be financially responsible for any compensation should you become injured during this study.If you have any concerns about your rights, how you are being treated or if you have

questions, want more information or have suggestions, please contact Eric Allen in the Office of Research Compliance at UNCG at (336) 256-1482. Questions, concerns or complaints about this project or benefits or risks associated with being in this study can be answered by Paul G. Davis who may be contacted at 336-334-3030 or pgdavis@uncg.edu

Are there any benefits to me for taking part in this research study?
For no charge, you may receive information concerning your cardiorespiratory fitness (VO_{2max}), body composition, blood pressure, and plasma lipid/lipoprotein profile.

Are there any benefits to society as a result of me taking part in this research?
Results from this study may provide new information regarding the protective effect of exercise against heart attack and stroke prevention, the number one and three causes of death to Americans.

Will I get paid for being in the study? Will it cost me anything?
There are no costs to you or payments made for participating in this study.

How will you keep my information confidential?
All information obtained in this study is strictly confidential unless disclosure is required by law. Subject data files are kept in a locked location and only anonymous data will be reported. Non-anonymous data will not be transferred via the internet or any other non-secure source. Persons working on the proposed project will take or have taken IRB-approved training on the proper handling of participant information.

What if I want to leave the study?
You have the right to refuse to participate or to withdraw at any time, without penalty. If you do withdraw, it will not affect you in any way. If you choose to withdraw, you may request that any of your data which has been collected be destroyed unless it is in a de-identifiable state.

What about new information/changes in the study?
If significant new information relating to the study becomes available which may relate to your willingness to continue to participate, this information will be provided to you.

Voluntary Consent by Participant:
By signing this consent form you are agreeing that you read, or it has been read to you,

and you fully understand the contents of this document and are openly willing consent to take part in this study. All of your questions concerning this study have been answered. By signing this form, you are agreeing that you are 18 years of age or older and are agreeing to participate, or have the individual specified above as a participant participate, in this study described to you by_____.

Signature: _____ Date: _____

APPENDIX E

PRESCREENING DATA FORM

Prescreening Data

Subject number_____

Date_____

Name_____ Weight _____kg _____lbs

Sex M F Height _____cm _____ins

Blood pressure BMI _____kg/m^2

Right arm

_____mm Hg _____mm Hg

Left arm

_____mm Hg _____mm Hg

Resting HR _____ bpm _____bpm _____ bpm _____bpm

Skinfolds

	1st measurement	2nd measurement	3rd measurement (if needed)	Average
Pectoral	_____mm	_____mm	_____mm	_____mm
Subscapular	_____mm	_____mm	_____mm	_____mm
Triceps	_____mm	_____mm	_____mm	_____mm
Midaxillary	_____mm	_____mm	_____mm	_____mm
Suprailiac	_____mm	_____mm	_____mm	_____mm
Abdominal	_____mm	_____mm	_____mm	_____mm
Thigh	_____mm	_____mm	_____mm	_____mm

Total= _____mm

% body fat = _____ %

APPENDIX F

RATING OF PERCEIVED EXERTION SCALE

Category Scale	
6	
7	Very, Very Light
8	
9	Very Light
10	
11	Fairly Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very, Very Hard
20	

APPENDIX G

$VO_{2\ MAX}$ DATA FORM

Name:_____ Age:_____ Subject #:_____

Date:_____

Pre-exercise HR:_____ Pre-Exercise BP:_____

Age Predicted Max HR:_____

Stage	**Stage Length**	**HR**	**RPE**	**VO_2**	**Comments**
1 4.5 mph					
2 4.8 mph					
3 5.2 mph					
4 5.6mph					
5 6.0mph					
6 6.3mph					
7 6.7mph					
8 7.1mph					
9 7.4mph					
10 7.8mph					
11 8.2mph					
12 8.6mph					
13 8.9mph					
14 9.3mph					
15					

9.7mph					
16 10.0mph					
17 10.4mph					
18 10.8mph					

Termination Time:_____ **Immediate-post VO_2:_____**

Immediate-post HR:_____ **Immediate-post BP:_____**

Reason for Termination:_____

APPENDIX H

FOOD DIARY DATA FORM

Please list ALL food and drink consumed during the three days before each experimental session. Use a separate sheet for each day and refer to the following examples for ideas on how specific you should be.

7:00am	Eggs (fried)	2 whole (jumbo)	180
		4 whites (jumbo)	75
	wheat bread	3 slices	200
	jelly (grape)	3 tsp.	?
	orange juice (conc.)	2 cups	200
11:00am	Rice (instant/white)	1 cup (dry)	300
	baked chicken	8 oz.	300
	(boneless/white meat)		
	olive oil	2 tbs.	240

Time	Food Source	Quantity (oz./cups)	Calories (if known)

Name:_____ Day:_____ Date:_____

APPENDIX I

SEVEN-DAY PHYSICAL ACTIVITY RECALL FORM

Subject #_____ **Treatment (circle) 60% 80%** **Date _____**

INTENSITY CLASS

0 = recreational exercise
1=conditioning exercise
2=brisk conditioning exercise
3=competitive strenuous exercise

SEVEN-DAY PHYSICAL ACTIVITY RECALL

1. How much of your leisure-time did you spend performing the following, specific activites last week. Please estimate the time spent in each activity and the intensity class during the week.

	Intensity class (0-3)	Time/week
Walking on work trips.....	_____	_____ h _____ min
Conditioning walking.....	_____	_____ h _____ min
Jogging.....	_____	_____ h _____ min
Cross-country skiing.....	_____	_____ h _____ min
Conditioning bicycling	_____	_____ h _____ min
Bicycling on work trips.....	_____	_____ h _____ min
Swimming.....	_____	_____ h _____ min
Gymnastics, dancing.....	_____	_____ h _____ min
Ball games.....	_____	_____ h _____ min
Gardening and snowshoveling.....	_____	_____ h _____ min
Hunting, picking up berries, gathering mushrooms.....	_____	_____ h _____ min
Fishing.....	_____	_____ h _____ min
Hobby crafts and repairs.....	_____	_____ h _____ min
Rowing.....	_____	_____ h _____ min
Forest work, wood cutting.....	_____	_____ h _____ min
Other, what:_____	_____	_____ h _____ min

2. Taking into account all leisure time during last week did the week differ considerably from a typical one for this time of the year?
   0 typical
   1 more activity as usual
   2 less activity as usual

3. How many kilometers did you perform following physical activites during your leisure time last week?

   Walking..... _____ km Skiing..... _____ km

   Jogging..... _____ km Swimming..... _____ km

   Bicycling..... _____ km

APPENDIX J

EXERCISE TREATMENT DATA FORM

Subject Name _____ Treatment Session 60%____ 80%____

Subject #_____

Subject fasted? _____

No exercise/significant PA for 48 hrs? _____

Current Body Weight _____kg _____lbs

$VO_{2\ max}$ _____

Target VO_2 _____

Timepoint (mins)	RPE	VO_2 ($ml/kg^{-1}/min^{-1}$)	HR (bpm)
5			
15			
25			